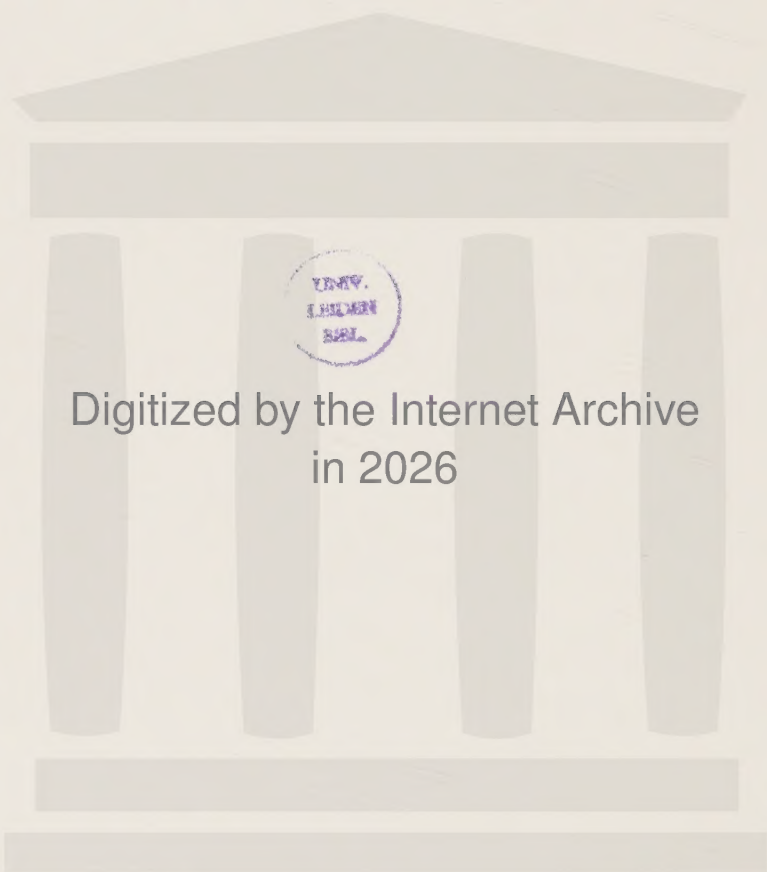


Effect of pneumococcal vaccination
on opsonization of pneumococci,
nasopharyngeal carriage of bacteria
and incidence of acute otitis media

Christer Rosén



Lund 1984



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Lund 1984

To Gunilla, Jeanna
and Josefine

The present thesis is based on the following papers, which will be referred to in the text by their roman numerals.

- I. Effect of pneumococcal vaccination on upper respiratory tract infections in children. Design of a follow-up study.
C. Rosén, P. Christensen, B. Hovelius & K. Prellner.
Scand J Infect Dis, Suppl 39: 39-44, 1983.
- II. Beneficial effect of pneumococcal vaccination on otitis media in children over two years old.
C. Rosén, P. Christensen, J. Henrichsen, B. Hovelius & K. Prellner.
Submitted to Int J Pediatr Otorhinolaryngol.
- III. A longitudinal study of the nasopharyngeal carriage of pneumococci as related to pneumococcal vaccination in children attending day-care centres.
C. Rosén, P. Christensen, B. Hovelius & K. Prellner.
Accepted for publication in Acta Otolaryngologica.
- IV. Nasopharyngeal carriage of bacteria in otitis-prone and non-otitis-prone children in day-care centres.
K. Prellner, P. Christensen, B. Hovelius & C. Rosén.
Accepted for publication in Acta Otolaryngologica.
- V. Opsonic and antibody responses to pneumococcal polysaccharide types 6A, 19F and 23F after vaccination of immunocompromised patients.
J. H. Braconier, F. Karup Pedersen, H. Odeberg & C. Rosén.
Scand J Infect Dis, in press.



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Introduction

FREQUENCY OF ACUTE OTITIS MEDIA AND OTHER UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN

Infections of the upper respiratory tract are very common, especially in childhood (Dingle et al, 1964; Chanock & Parrott, 1965; Monto & Ullman, 1974).

Children attending day-care centres are more prone to acquire upper respiratory tract infections than children otherwise cared for (Hesselvik, 1949; Ståhlberg, 1980). Approximately 90% of all acute diseases in children at day-care centres are upper respiratory tract infections (Beem, 1969).

Acute otitis media (AOM) is a common bacterial disease in children (Brownlee et al, 1969; Howie, 1975). 30% of Swedish children have been found to have an AOM episode during their first year (Ingvarsson, 1982), and children attending day-care centres to suffer from AOM more frequently than those cared for in their own homes or in family-care homes (Strangert, 1977; Ingvarsson, 1982; Vinther et al, 1982). Despite antimicrobial treatment the incidence of AOM is high (Howie, 1975; Teele et al, 1980) and the elimination of nasopharyngeal bacteria in AOM by antimicrobials has been limited or transient (Herberts et al, 1971; Rosén et al, 1983), although serious complications are less common (Hamberger, 1942; Bass et al, 1967; Howard et al, 1976).

In 1982, 149,000 Swedish children (or 23% of those born between 1975 and September 1982) attended day-care centres (Statistiska centralbyrån, S 1983:12). The corresponding figure for the region of Lund (locus of the present study) was 31.2% (Statistiska centralbyrån, S 1983:6).

It was recently reported that the average absence from Swedish day-care centres due to disease was 12.3% (Socialdepartementet, DsS 1978:15). Apart from discomfort to the children, many parents must absent themselves from work to take care of their children, which is a pronounced socio-economic problem.

MICROBIOLOGY OF ACUTE OTITIS MEDIA AND OTHER UPPER RESPIRATORY TRACT INFECTIONS

It is well established that, at least primarily, upper respiratory tract infections are largely caused by viruses (Paterson, 1980). With the exception of β -haemolytic streptococci, potentially pathogenic bacteria causing infection in

the upper respiratory tract are looked upon merely as secondary invaders (Chanock & Parrott, 1965). In a longitudinal study at a day-care centre, the incidence of AOM increased in children with viral respiratory infections (Henderson et al, 1982).

The pneumococcus is the bacterial pathogen most frequently isolated in the upper respiratory tract in both healthy and sick children. In AOM, pneumococci are isolated from middle ear fluid in 30-50% (Howie et al, 1970; Kamme et al, 1971a; cf. Klein, 1980; Qvarnberg, 1981). The types/groups 6, 14, 19 and 23 are responsible for more than half of all pneumococcal AOM episodes (Kamme et al, 1970; Sloyer et al, 1974; Austrian et al, 1977).

Haemophilus influenzae is the second most frequent bacteria, isolated in 20-30% of the cases of AOM in preschool children (cf. Klein, 1980). In some studies, the nasopharyngeal carrier rates of H.influenzae in healthy children are close to those of pneumococci (Masters et al, 1958). The strains that cause AOM are predominantly non-typable (Kamme et al, 1971b; Karma et al, 1983). Among capsulated strains, type b in particular has been frequently reported (cf. Sell & Karzon, 1973; cf. Giebink & Quie, 1978). H.influenzae has also been a frequent finding in sinusitis in children (Andréasson et al, 1982).

Branhamella catarrhalis is a common inhabitant of the upper respiratory tract, and has been found in about 10% in purulent middle ear effusions (Coffey et al, 1966, 1967; Kamme et al, 1971a). Together with the isolation of B.catarrhalis in patients with AOM, an increased specific antibody concentration has been demonstrated in middle ear effusions (Leinonen et al, 1981). This supports the view that B.catarrhalis should be looked upon as a pathogen in AOM.

β -haemolytic streptococci are present in about 20-70% of tonsillitis-pharyngitis episodes (Glezen et al, 1967; Cars & Forsum, 1975; Hovellius et al, 1983), but are seldom isolated in AOM nowadays (cf. Klein, 1980; Qvarnberg, 1981).

CARRIAGE OF BACTERIA IN THE NASOPHARYNX AND THROAT

Healthy children

The frequency with which potentially pathogenic bacteria are isolated from the nasopharynx or throat in asymptomatic children ranges from 15% to 90% (Masters et al, 1958; Willard & Hansen, 1959; Box et al, 1961; Norstedt, 1967; Branefors-Helander et al, 1972; Ingvarsson et al, 1982). In some studies, cultures have been obtained both from the nasopharynx and throat, showing a high agreement in the frequency of bacterial strains between the two sources (Masters et al, 1958; Box et al, 1961; Hendley et al, 1975; Gray et al, 1980). Some children carry the same bacterial strains for long periods

and yet do not fall ill (Yang, 1941; Dunlap & Harvey, 1956; Loda et al, 1975).

Children with AOM

In many investigations of AOM, samples for culture have been collected simultaneously both from the nasopharynx and from ear effusions, and a good correlation has been found between the bacterial strains isolated (Howie & Ploussard, 1971; Kamme et al, 1971a; Branefors-Helander et al, 1972; Schwartz et al, 1979). 70-80% of nasopharyngeal cultures from children with AOM have shown the presence of potentially pathogenic bacteria (Howie & Ploussard, 1971; Nylén, 1975; Puhakka et al, 1979; Luotonen, 1982).

Carriage of pneumococci and its relation to upper respiratory tract infection in children

Carriage rates of pneumococci in healthy individuals have been found to be highest at early ages - in preschool children about 30-45% (Masters et al, 1958; Dowling et al, 1971; Loda et al, 1975; Gray et al, 1980), decreasing with age to about 10-20% in adults (Hendley et al, 1975). Rates are higher in adults in families with preschool children than in those without (Hendley et al, 1975).

The acquisition of pneumococci seems to be promoted by environmental influences, such as living under crowded conditions in families (Brimblecombe et al, 1958), in orphanages (Straker et al, 1939) or in day-care centres (Yang, 1941; Loda et al, 1975).

Seasonal fluctuation in asymptomatic carriage has been noted by many authors, the rates being lower during summer months but otherwise consistent, with occasional peaks in the winter (Hendley et al, 1975; Loda et al, 1975; Gray et al, 1980).

The pneumococcal types/groups most frequently isolated in healthy children are 3, 6, 14, 19 and 23 (Masters et al, 1958; Dowling et al, 1971; Loda et al, 1975; Gray et al, 1980). Loda and colleagues (1975) observed that especially types/groups 6, 19 and 23, which had been carried for long periods, were also frequently reacquired.

There is a general increase in carriage rates of bacteria, including pneumococci, among people with common colds (Kneeland & Dawes, 1932; Straker et al, 1939; Brimblecombe et al, 1958), and the spread of pneumococci has been shown to occur in conjunction with rhinovirus infection (Gwaltney et al, 1975). However, the reason why asymptomatic carriers may suddenly succumb to pneumococcal infection is not yet known.

The types/groups of pneumococci usually found in healthy carriers appear to be those commonly found in different kinds

of pneumococcal infections (Siegel et al, 1978; Gray et al, 1979).

Gray and co-workers (1980) found that pneumococcal types/groups that were carried for prolonged periods in children were causative agents only rarely, and that 80% of pneumococcal infections were due to newly acquired types. In conflict with this, Austrian and colleagues (1977) observed that nasopharyngeal carriage of a type often occurred long before an episode of AOM caused by that type, and that carriage might persist long after recovery from the infection without causing a new one.

PNEUMOCOCCI AND OPSONIC REQUIREMENTS

The pneumococci are gram-positive diplococci, possessing a capsule of polysaccharide (cf. White, 1979; cf. Jawetz et al, 1982). The capsular polysaccharide is immunologically distinct for each of the types, more than 80 types at present being recognized on the basis of the antigenic properties of the capsule (Lund & Henrichsen, 1978; Schiffman, 1981).

The antigenic similarity between certain types is reflected in the widely used Danish system of nomenclature for pneumococci (Lund & Henrichsen, 1978), where related types are arranged in groups.

It is not known why most pneumococcal infections are caused by a limited number of pneumococcal types (Austrian et al, 1977; Siegel et al, 1978; Gray et al, 1980).

The most important virulence factor is considered to be the capsular polysaccharide, which has antiphagocytic properties but lacks toxicity (cf. Austrian, 1981).

The ultimate purpose of the host defence against pneumococci is to eliminate infecting bacteria by phagocytosis. To achieve this requires a coordinated interaction of humoral factors, such as immunoglobulins and complement (Brown et al, 1982), and phagocytosing cells (cf. Stossel, 1974).

Specific immunity to bacteria may be induced in the mucosa by locally produced immunoglobulins (Bernstein et al, 1974; Mogi et al, 1974; Hjort Sørensen, 1982) or by diffusion or transudation of serum antibodies (cf. Hanson et al, 1983).

The coating, opsonization, of the pneumococci by specific anticapsular antibodies and complement cleavage fragments, especially C3b, promotes phagocytosis (Johnston et al, 1969; Winkelstein, 1973).

To obtain C3b, the complement system must be activated, and immune-complexes are its most important activators (Augener et al, 1971). Specific anticapsular antibodies are known to form immune-complexes with pneumococcal antigen, and thus

activate the complement system (Winkelstein, 1973).

This complement activation proceeds like a chain reaction via the classical pathway. C3b can also be formed in the absence of specific antibody, via the alternative pathway of complement activation (cf. Winkelstein, 1981).

For phagocytosis to occur, the pneumococci must come into contact with the granulocytes. Chemotaxis, the directed migration of granulocytes from the circulation to the site of microbial invasion (cf. Wilkinson, 1982), is induced by several substances derived from the inflammatory reaction. The major attractants are cleavage products of the fifth component of complement (Ward et al, 1965; Snyderman et al, 1971; Fernandez et al, 1978), for which the granulocytes have specific receptors (Chenoweth & Hugli, 1978).

The granulocyte has surface receptors for recognition of the specific antibody and of the complement component C3b (cf. Stossel, 1974), opsonized pneumococci being easily engulfed and killed by the granulocytes (cf. Klebanoff & Clark, 1978).

The requirement of antibodies for efficient opsonization of pneumococci is demonstrated by the in vitro findings that granulocyte phagocytosis of the pneumococcal types/groups 6, 18, 23 and 25 was reduced in hypogammaglobulinaemic serum (Giebink et al, 1977).

Without complement, however, phagocytosis of pneumococci occurs only slowly, even when sufficient amounts of immunoglobulins are present (Brown et al, 1982).

In vivo, the importance of specific anticapsular antibodies in defence against pneumococci is stressed by the fact that patients with hypogammaglobulinaemia are frequently infected by pneumococci (Rosen & Janeway, 1966), and that specific pneumococcal antisera, often used in the pre-antibiotic era, had a therapeutic effect when given early in severe pneumococcal infections (cf. Heffron, 1979). Furthermore, replacement therapy with gammaglobulin reduces pneumococcal infections in patients with hypogammaglobulinaemia (cf. Hanson, 1983).

The development of type-specific pneumococcal antibodies may be induced by carriage (Gwaltney et al, 1975), infection (Sloyer et al, 1974; Branefors et al, 1980), vaccination (Borgoño et al, 1978), or a combination of those. Antibodies against pneumococci are often present in healthy children, but in low amounts as compared with those found in healthy adults (Borgoño et al, 1978).

In otitis-prone children, Prellner and co-workers (1984) found low amounts of serum antibody against types 6A, 19F and 23F.

PNEUMOCOCCAL VACCINATION AND ANTIBODY RESPONSE

The pneumococcal vaccine currently used is composed of capsular polysaccharides from 14 different types of pneumococci, 50 µg per dose of each pneumococcal polysaccharide usually being used. In small children, 25 µg per dose of each polysaccharide has been used (Mäkelä et al, 1980; Sloyer et al, 1981; Douglas et al, 1983), apparently with much the same immunogenic effect as 50 µg per dose (Cano et al, 1983). Vaccination with only one pneumococcal polysaccharide has shown an increase of antibody concentration similar to that obtained when the pneumococcal type in question was included in a polyvalent vaccine (cf. Schiffman, 1981). The types included in the vaccine are responsible for about 80% of documented pneumococcal disease in the USA (Austrian et al, 1976).

Immunization with pneumococcal vaccine has been shown to result in a reduced increase of antibody concentrations in children than in adults (Borgoño et al, 1978; Cowan et al, 1978).

Certain pneumococcal types/groups, which most commonly cause pneumococcal AOM in children (i.e., 6, 19 and 23) have been identified as poor immunogens during the first years of childhood (Douglas et al, 1983). By contrast, immunization with type 3 generally results in high amounts of antibodies, even in children (Cowan et al, 1978; Sell et al, 1981; Douglas et al, 1983).

The amounts of specific pneumococcal antibodies needed to prevent different infections are not yet known (cf. Hilleman et al, 1981). 300 ng of Ab N/ml of type-specific antibody has been proposed as the minimum to ensure protection against serious pneumococcal infections (cf. Landesman & Schiffman, 1981). This value, however, is based upon antibody concentrations at vaccine-failures and concerns adults with pneumonia or septicaemia (cf. Landesman & Schiffman, 1981).

In vaccinated adults antibody concentrations, at least half as great as those obtained 4 weeks after vaccination, were still present 4 years later (Mufson et al, 1983). Although data about children are sparse, in one study children between 2 and 12 years of age were found to have retained their antibody concentrations well, with only a slight decline at 21 months after the vaccination (Vella et al, 1980).

Adverse reactions to the pneumococcal vaccine are usually mild and generally limited to tenderness at the injection site (Borgoño et al, 1978). On rare occasions the local reaction is more severe and accompanied by fever (Uhl et al, 1978; Semel & Seskind, 1979).

When a second dose has been given, an increased number of local and systemic reactions have been reported among adults (Borgoño et al, 1978), but not in children (Douglas et al,

1983).

After revaccination little or no "booster" effect has been observed, either in children (Sloyer et al, 1980; Douglas et al, 1983), or in adults (cf. Hilleman et al, 1978).

EFFECT OF PNEUMOCOCCAL VACCINATION ON INFECTIONS

Historical aspects

Pneumococcal vaccine, composed of whole pneumococcal cells, was first tried out in South Africa to prevent pneumonia among mine workers (Wright et al, 1914).

In the 1920s and 1930s, the antigenicity of pneumococcal capsular polysaccharides was demonstrated in experimental animals (Schiemann & Casper, 1928) and later in humans (Francis & Tillett, 1930), but trials of vaccines, composed of pneumococcal polysaccharides, failed to provide clear evidence of its efficacy (Ekwurzel et al, 1938). However, a few years later MacLeod and co-workers (1945) could demonstrate the efficacy of a quadrivalent vaccine in the prevention of pneumococcal pneumonia and in the elimination of pneumococci from the nasopharynx in military populations.

With the introduction of antimicrobial treatment - first the sulphonamides (Domagk, 1935) and then penicillin (Chain et al, 1940) - the problems with pneumococcal infection were thought to have been solved, and interest in pneumococcal vaccine waned (cf. Austrian, 1981). Despite the striking effect of antibiotics, however, their promise has not been fulfilled; instead:

- a) The incidence of pneumococcal disease remained unaffected even if the clinical outcome improved (cf. Austrian, 1975; World Health Statistics Annual Vol 1, WHO 1976; cf. Kaijser, 1979).
- b) Certain types of patients (such as those that have been splenectomized) are highly susceptible to fatal pneumococcal infections (Barrett-Connor, 1971; Singer, 1973).
- c) Pneumococcal strains have developed that are resistant to antibiotics (cf. Ward, 1981).

This has emphasized the importance of a preventive rather than a purely therapeutic approach to pneumococcal infections, and led to renewed interest in the development of a pneumococcal vaccine (Austrian & Gold, 1964). Field trials with various numbers of capsular polysaccharides provided evidence supporting the efficacy of the vaccines, and resulting in the issue of licences in several countries.

Adults

Successful contemporary studies in adults on the efficacy of pneumococcal vaccines in protection against pneumonia have been carried out in susceptible populations such as mine

workers (Austrian et al, 1976; Riley et al, 1977; Smit et al, 1977), and in special groups of patients at high risk for pneumococcal infection, for example, because they have had a splenectomy (Ammann et al, 1977).

Children

There are a limited number of studies on the efficacy of pneumococcal vaccination in children, (Ammann et al, 1977; Mäkelä et al, 1980; Riley et al, 1981; Sloyer et al, 1981; Teele et al, 1981; Wright et al, 1981).

Ammann and colleagues (1977) reported that after vaccination with an octavalent vaccine in children, 2 years of age and above, with sickle-cell disease or splenectomy or both, the antibody response was satisfactory (Ammann et al, 1977). Over a 2-year period of observation none of the 77 vaccinees contracted septicaemia due to types present in the vaccine, while 8 of the 106 control children did.

Riley and co-workers (1981) found the incidence of lower respiratory tract infections in children living in the highlands of New Guinea to have been reduced by 37%, provided they had been at least 17 months of age when immunized.

Vaccination trials concerning the prophylactic effect on upper respiratory tract infections are quite rare, and have been exclusively focused on AOM (Mäkelä et al, 1980; Sloyer et al, 1981; Teele et al, 1981; Wright et al, 1981).

These investigators recruited their patients after they had suffered at least one, but often more than one AOM episode. The children received an octavalent vaccine (Sloyer et al, 1981; Teele et al, 1981; Wright et al, 1981), or a 14-valent vaccine (Mäkelä et al, 1980; Wright et al, 1981), as the target vaccines, while other bacterial polysaccharide vaccines (Mäkelä et al, 1980; Sloyer et al, 1981; Teele et al, 1981), or no vaccination at all (Wright et al, 1981) were used in controls. The effect of vaccination was evaluated as a vaccine-associated reduction in pneumococcal AOM. In the study of Mäkelä and co-workers (1980), children that had been vaccinated between the ages of 7 and 83 months suffered from fewer AOM episodes than did control children, and a 56% reduction of AOM episodes was shown. However, since no reduction with regard to pneumococcal otitis media caused by group 6 was found, these data were omitted. The results have been questioned by Filice (1980), i.e. since exclusion of this type, a frequent cause of infection, biased the statistical calculations.

In children aged 5 to 21 months when vaccinated, Teele and co-workers (1981) demonstrated fewer episodes of AOM due to pneumococcal types present in the vaccine, although the number of children studied was small and there was no difference in the overall incidence of AOM between vaccinees and controls.

In contrast, neither Sloyer and colleagues (1981), nor Wright and co-workers (1981), were able to show any reduction of vaccine-type pneumococcal AOM in children vaccinated below 2 years of age.

Aims of the investigation

- I. To assess the effect of pneumococcal vaccination on the overall incidence of AOM and other upper respiratory tract infections in children.
- II. To study the nasopharyngeal carriage of pneumococci and the impact of vaccination in children attending day-care centres.
- III. To compare the carriership of pneumococci, Haemophilus influenzae and Branhamella catarrhalis in otitis-prone children and in children with no history of AOM.
- IV. To investigate the effect of pneumococcal vaccination on antibody concentration and opsonic activity against pneumococcal types 6A, 19F and 23F.

Incidence of acute otitis media, other upper respiratory tract infections and carriage of bacteria in children (I, II, III, IV) - Effect of pneumococcal vaccination (I, II, III)

STUDY POPULATIONS AND METHODS

Design of the study

The study was designed as a double-blind, pair-matched trial to determine the effect of immunization with a pneumococcal vaccine, Pneumovax®, on the frequency of otitis media, other upper respiratory tract infections, and on nasopharyngeal carriage in children during a 2-year follow-up period.

It was planned to recruit 400 children of whom at least 100 were less than 2 years of age. For this purpose parents of 4,000 children attending 66 day-care centres in the Lund district were given verbal and written descriptions of the project. Information concerning the medical history and social environment of the child was collected from a questionnaire answered by parents, after their written consent had been obtained for their children to be included in the study.

Children between the ages of 6 months and 5 years were recruited with the following exceptions:

Children with cleft palates; with chronic or progressive diseases; with any disease treated with ACTH, corticosteroids, irradiation, alkylating agents or antimetabolites; with known immunological deficiencies or hypersensitive to any component in the vaccine.

The design of the study was approved by the Ethics Committee of the University of Lund.

Study populations

In all, 405 children were included in the study, being allocated to one of the following groups according to age and previous history of AOM media:

1. <2 years of age with no previous episode of AOM (40 children)
2. <2 years of age with previous episode(s) of AOM (52 children)
3. 2-5 years of age with no previous episode of AOM (94 children)
4. 2-5 years of age with previous episode(s) of AOM (219 children).

The children were pair-matched according to the stratification above, one child in each pair receiving vaccine and the other saline as placebo. In all, 198 children were vaccinated and 207 served as controls; 16 children (4 vaccinees, 12 controls) could not be pair-matched.

There were no significant differences between the groups of vaccinated and control children as to age, sex or medical history (I). Number and age of family members, as well as housing conditions were similar in the two groups (I).

Particular attention was paid to the following subpopulations among the children recruited to the study:

To study epidemiological data of pneumococci in day-care centres (III), 160 children were selected from whom specimens were taken 10 times or more with an interval of less than 45 days between any two samplings.

To compare the nasopharyngeal carriage of bacteria in otitis-prone and non-otitis-prone children, two groups of children were selected among the 253 children born 1977-1979 (IV).

Children were placed in the otitis-prone group when they fulfilled the criteria of one of the following categories:

- under treatment with ventilation tubes due to recurrent AOM (>5 episodes) at enrollment, and had experienced at least one AOM episode during the 2-year follow-up period; or
- had a history of >3 AOM episodes at enrollment, and during the follow-up at least 2 AOM episodes; or
- had a history of 1-2 AOM episodes at recruitment, and had experienced at least 3 episodes of AOM during the follow-up period.

Thus defined, 26 children (10%) were otitis-prone.

Children recruited to the non-otitis-prone group had no history of AOM or pneumonia, not more than one episode of acute tonsillitis or bronchitis, and less than 4 episodes of common cold per year. During the 2-year follow-up period, they had no episode of AOM or pneumonia, not more than one acute visit to a doctor and/or one period of absence from the day-care centre due to respiratory tract infection. Thus, the non-otitis-prone children had few other upper respiratory tract infections either.

The non-otitis-prone group comprised 36 (14%) children.

Immunization

Of children more than 2 years old one child in each pair received one injection of 0.5 ml Pneumovax® (containing 50 µg of each capsular polysaccharide per dose), and the other a placebo of saline with the same preservatives as the vaccine. To children less than 2 years old was given 0.25 ml (containing 25 µg of each capsular polysaccharide per dose) of vaccine or saline. The injections were given subcutaneously in the deltoid area.

The vaccine contained capsular polysaccharides from types 1, 2, 3, 4, 6A, 7F, 8, 9N, 12F, 14, 18C, 19F, 23F and 25 (Danish nomenclature).

Any side effects of vaccination were registered by parents on a special form.

Clinical follow-up

Any children needing medical care during the 2-year follow-up period were asked to attend a special clinic run by two of the investigators who were ENT-specialists and available for consultation one hour daily on weekdays. Children seeking medical care at other times were examined, and treated when necessary, by their own general practitioners or by the duty doctor at the Ear, Nose and Throat Clinic at the University Hospital of Lund. At each consultation the usual medical record was supplemented by a special form reporting disease history, objective findings, diagnosis and therapy. Any therapy given at such consultations followed normal practice. 80-90% of the acute visits were to ENT-specialists (I, II, IV), otomicroscopy being then routinely performed.

Bacteriological specimens

Each day-care centre was visited by a special trained nurse once every month except July and August. Specimens were collected from the nasopharynx and throat of all children present that day, except those taking antibiotics. Such specimens were also obtained at every acute visit.

Pneumococci, Haemophilus influenzae and β -haemolytic streptococci were identified using conventional methods. Pneumococci were serotyped by the Quellung reaction (Neufeld, 1902; Lund & Henriksen, 1978). β -haemolytic streptococci were serologically grouped and group A streptococci T-typed (Christensen et al, 1973; Ivarsson & Christensen, 1977). Branhamella catarrhalis was identified as described by Kamme (1970).

Statistical methods

The χ^2 test was used for statistical analysis.

THE EFFECT OF PNEUMOCOCCAL VACCINATION (I, II, III)

Effect on bacterial carriage

The total nasopharyngeal carriage rates for pneumococci in specimens taken on scheduled occasions were similar for vaccinees, 31.7%, and controls, 32.1%. Neither were there any differences in pneumococcal carriership when vaccinees and controls were compared, regardless of whether they were under or over 2 years of age. Notably, even when pneumococci of group 6 - known to be poor immunogens in children (Cowan et al, 1978; Douglas et al, 1983) - were excluded, the nasopharyngeal carriage rates for the other vaccine types/groups did not differ between vaccinees and controls.

Effect on AOM

In children less than 2 years old when vaccinated, episodes of AOM were as frequent among the 48 controls (56 episodes) as among the 44 vaccinees (67 episodes) ($p > 0.05$).

101 of the 313 children, 2-5 years old when given vaccine/saline, accounted for 177 visits due to AOM, 75 episodes occurring in vaccinees and 102 in the controls. Thus, there was a reduction of episodes per child by 24% in the vaccinees as compared with the controls ($p < 0.05$).

However, the number of first AOM episodes did not differ between vaccinees and controls (47 and 54 episodes, respectively, $p > 0.05$). The reduction in AOM episodes in the vaccine group was due to a reduction in the number of second and subsequent attacks (40% decrease in vaccinees, $p < 0.05$). During the first postvaccinational year, a 30% decrease of AOM episodes was found in vaccinees as compared with controls ($p < 0.05$). During the second postvaccinational year, the number of AOM episodes were lower in the vaccinees, 2-5 years of age, than in the controls, but this difference was not significant ($p > 0.05$).

In children with no experiences of otitis media at enrollment, 7 episodes occurred in 47 vaccinees and 15 in 47 controls. This numerical difference was, however, not statistically significant ($p > 0.05$).

Effect on other upper respiratory tract infections (I)

The results of the 1½ year follow-up were published in paper I. In children 2-5 years old at vaccination, a significantly reduced number of visits due to tonsillitis and pharyngitis were registered in the vaccinees as compared with the controls. The frequency of visits due to other causes did not differ between vaccinees and controls. These findings remained throughout the entire study. Data at the end of the 2-year follow-up period are given in Table I. Results concerning children under 2 years of age at vaccination are also

Table I. Number of certain upper respiratory tract infections other than AOM in 198 vaccinees and 207 controls.

Diagnosis	Children <2 years		Children 2-5 years	
	vaccinees n=44	controls n=48	vaccinees n=154	controls n=159
Rhinitis	63	62	65	83
Pharyngitis	20	16	11 ^{a)}	30 ^{a)}
Tonsillitis	20 ^{b)}	6 ^{b)}	13 ^{c)}	32 ^{c)}

a) $p < 0.01$

b) $p < 0.01$

c) $p < 0.01$

presented. In this age group the incidence of tonsillitis and pharyngitis was higher in vaccinees than in controls ($p < 0.01$). In the group of children under 2 years of age when given vaccine or placebo, visits due to tonsillitis and pharyngitis were more frequent than in 2-5 year-old children ($p < 0.01$).

Side effects (I)

Side effects of vaccination/placebo injections were reported in 31% of the vaccinees and 1.5% of the controls. Adverse reactions were generally limited to mild pain and tenderness at the site of injection. 3% of the vaccinees reported fever, defined as a body temperature above 38°C , in association with the local reaction. No severe reactions were registered.

Conclusions

Pneumococcal vaccination did not affect nasopharyngeal carriage of pneumococci. Pneumococcal vaccination reduced the number of AOM episodes in children vaccinated between the ages of 2 and 5. In this age group a reduced rate of tonsillitis and pharyngitis was also seen following vaccination.

EPIDEMIOLOGICAL STUDIES ON THE NASOPHARYNGEAL CARRIAGE OF PNEUMOCOCCI (III)

Since vaccination did not influence the nasopharyngeal carriage of pneumococci, vaccinees and controls were considered together in the epidemiological studies.

The number of children attending the day-care centres included in the study varied between 12 and 96, half of the centres (33) being attended by 45 children or more (mean: 59 children). 33 children on average attended day-care centres with less than 45 children. At most, 40% of the children at any day-care centre were enrolled in the study. Of the total number of children participating in the study (405), 252 attended centres with 45 children or more.

The nurse responsible for the scheduled visits collected specimens from the nasopharynx and throat of the children on 3,418 occasions. On average, 8.4 paired nasopharyngeal and throat specimens per child were obtained. In addition to occasions when children were on antibiotics, specimens sometimes could not be taken due to absence from the day-care centre, absence being due to illness, holidays or having to stay at home when their mother had just had another baby.

A subpopulation of frequently sampled children was selected to study acquisition, reacquisition and duration of carriage of pneumococci (see above: Study population, subpopulations).

Certain epidemiological aspects of the spreading of pneumococci were studied only in day-care centres where at least 12 children were enrolled in the study.

Relation of carriage to age, season of the year and size of day-care centre

Pneumococci were found in 1,091 of 3,418 nasopharyngeal cultures taken on scheduled occasions (31.9%). Children of under 2 years at the time of sampling carried pneumococci more frequently (46.2%) than did children above 2 years of age (30.6%; $p < 0.001$).

During 1981 the monthly proportions of the carriers varied from 25% (January) to 42% (April), and those for January and September were significantly lower than those for April and October ($p < 0.05$).

Children in day-care centres with more than 45 attendees carried pneumococci more frequently than those in smaller ones (34.2% and 28.7%, respectively; $p < 0.01$). No correlation was found between carriage and the number of members in the participant child's family.

Acquisition, reacquisition and duration of carriage

Acquisition of a given pneumococcal type in a child for the first time was registered 336 times in the subpopulation of 160 children from whom a sufficient number of culture samples were obtained (see above). One child reacquired 7 different types/groups, while no pneumococci at all were found in 19 children.

24 instances were found on reacquisition of a previously carried type/group after two negative cultures, but no child reacquired the same type more than once. Groups 6, 23 and 19 were most frequently reacquired, while other types seldom reappeared.

Among the four most common types/groups of pneumococci, those belonging to group 6 pneumococci were carried significantly longer than groups 19 and 23 ($p < 0.05$), but not longer than type 14 ($p > 0.05$). These types/groups were cultured on one occasion only in 171 instances, but were carried for 3 months or more in 20 children.

Spreading within day-care centres

After the first appearance of a pneumococcal type belonging to group 6 in a child, this type was isolated on average in 3.6 children in the respective day-care centre at the following monthly visit. The corresponding figures for types/groups 14, 19 and 23 were 2.6, 2.8 and 3.2. The average figures for the second month ranged between 0.3 (types/groups 6, 14, 19) and 0.8 (group 23).

Conclusions

Pneumococci were frequently encountered among the children, especially in those under 2 years of age.

In day-care centres attended by 45 children or more the carriage rate of pneumococci was significantly higher than in centres with less than 45 children.

Spreading within centres was common but of short duration. Pneumococci of group 6 were carried most frequently and for longer periods than other types/groups.

NASOPHARYNGEAL CARRIAGE OF PNEUMOCOCCI, HAEMOPHILUS INFLUENZAE AND BRANHAMELLA CATARRHALIS IN OTITIS-PRONE AND NON-OTITIS-PRONE CHILDREN (IV)

From 253 children who were 1.5-3.5 years old at the beginning of the study and 3.5-5.5 years old at the end, one group of otitis-prone and another of non-otitis-prone children (see above) were selected for special study.

The carriage rates were studied of pneumococci, H.influenzae and B.catarrhalis when asymptomatic and at episodes of AOM.

Vaccinees and controls were considered together since pneumococcal vaccination did not affect isolation frequencies of pneumococci, H.influenzae or B.catarrhalis (II, III). Besides, there were no significant differences between the proportion of those vaccinated in the otitis-prone and non-otitis-prone groups.

Carriage on scheduled occasions and at AOM episodes

Both pneumococci, H.influenzae and B.catarrhalis were found as frequently in non-otitis-prone children as in asymptomatic otitis-prone children. At AOM episodes only B.catarrhalis was found significantly more often than in cultures taken on scheduled occasions (Fig. 1).

Isolation
frequency
(percent)

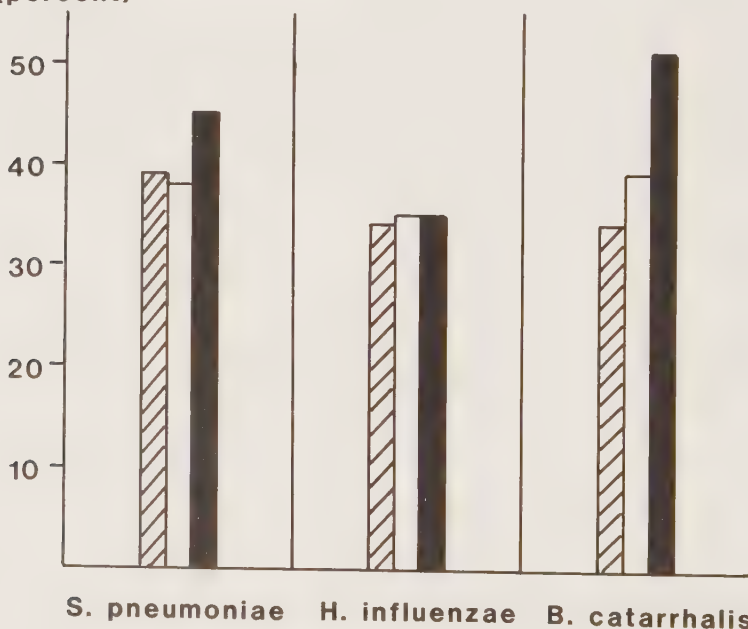


Fig. 1. Nasopharyngeal isolation frequencies of S.pneumoniae, H.influenzae and B.catarrhalis in 335 specimens from 36 non-otitis-prone children (▨), in 268 specimens from 26 otitis-prone children when asymptomatic (□) and in 84 specimens obtained at episodes of AOM (■).

The isolation frequencies of pneumococcal types/groups 6, 23, 19, 14, 11 and 18, which were those most commonly found, were similar in non-otitis-prone and in otitis-prone children, whether the cultures were taken at the asymptomatic state or at AOM episodes.

Pneumococci of group 6 was the most common type found and accounted for 29% of the total number of isolates. The second and third most common were groups 23 and 19, found in 12% and 10% of the isolates, respectively.

The duration of carriage of a given pneumococcal strain did not differ between non-otitis-prone and otitis-prone children.

Carriage in relation to age

The results of scheduled cultures obtained when the children were 2-3 years old were compared with those of the specimens taken at the age of 3-4 years.

The isolation frequencies for pneumococci, H.influenzae and B.catarrhalis were similar in both age groups for non-otitis-prone children. Among 2-3 year-old asymptomatic otitis-prone children, H.influenzae was detected in 41% and in 3-4 year-old children in 23% ($p < 0.05$). B.catarrhalis showed similar differences between the two age groups, 49% and 23% ($p < 0.001$), whereas the isolation frequencies for pneumococci were about the same. Among the children 3-4 years of age, B.catarrhalis was found in 23% of the specimens in asymptomatic otitis-prone children as compared with 43% in non-otitis-prone children, a significant difference ($p < 0.05$).

Conclusions

Carriership of pneumococci, H.influenzae and B.catarrhalis in the nasopharynx was at least as common in non-otitis-prone children as in asymptomatic otitis-prone children. In 3-4 year-old children B.catarrhalis were isolated more often in non-otitis-prone children than in asymptomatic otitis-prone children. Thus, the presence of potential pathogens per se did not seem to increase the risk of developing AOM.

The frequency with which the different pneumococcal types//groups were isolated at AOM episodes appeared to be proportional to the rates of carriage in asymptomatic children.

Effect of pneumococcal vaccination on antibody concentration and opsonic activity against pneumococcal types 6A, 19F and 23F (V)

MATERIALS AND METHODS

Adult patients (15-76 years), highly susceptible to pneumococcal septicaemia (cf. Landesman & Schiffman, 1981), were immunized subcutaneously with 0.5 ml of a 14-valent pneumococcal vaccine, containing 50 µg of each capsular polysaccharide (Pneumovax®). The correlations between the increase of serum antibody concentrations against the pneumococcal types 6A, 19F and 23F, and both the enhancement of opsonization as measured by phagocytic killing, and the induction of their chemotactic activity were studied in vitro.

Patient sera

The sera studied were collected both before and after immunization from 8 non-splenectomized patients with malignant diseases and 25 splenectomized patients, and were stored at -80°C until analysed. Splenectomy had been performed because of trauma in 8 patients, non-malignant diseases in 9 patients and malignant diseases in 8 patients.

Granulocytes

Human granulocytes were separated from peripheral blood of healthy volunteers, using Isopaque-Ficoll (Böyum, 1968). After lysis of red cells with 0.87% NH₄Cl, the granulocytes were washed and suspended in HEPES-HBSS (Odeberg et al, 1975).

Bacteria

Pneumococci of types 6A, 19F and 23F were maintained on blood agar and renewed monthly from stock cultures. For the experiments pneumococci were used that had been harvested in the logarithmic growth phase (Braconier & Odeberg, 1982) and adjusted to the desired concentration by a spectrophotometric method.

Antibody measurements

Antibodies against pneumococcal capsular polysaccharides of types 6A, 19F and 23F were measured by an enzyme-linked immunosorbent assay (ELISA) (Karup Pedersen & Henrichsen, 1982).

Phagocytic killing of pneumococci

About 5×10^6 bacteria were incubated for 30 minutes at 37°C with 10^6 granulocytes in serum (Braconier & Odeberg, 1982) unless otherwise indicated, diluted to 16%, in a total volume of 250 μl . Surviving bacteria were quantified by colony counting.

Granulocyte chemotaxis

Chemotaxis of granulocytes from healthy individuals was studied with an under-agarose technique, modified from Nelson and co-workers (1975). Approximately 10^5 of each *S.pneumoniae* type (6A, 19F and 23F) were incubated in 12.5% of prevaccination or postvaccination sera for 4 minutes at 37°C . After heat inactivation at 56°C for 30 minutes followed by centrifugation, the supernatants were used as chemo-attractants to the granulocytes. The chemotaxis was quantified after 3 hours incubation by measuring the distances of migration of the granulocytes towards the supernatants.

RESULTS AND CONCLUSIONS

A 2-fold or greater increase in antibody concentration and a significantly enhanced opsonization was found in about 50% of the tests, irrespective of which pneumococcal type was studied. A close correlation was found between increase of antibody concentration and enhancement of opsonization. Patients whose antibody concentration increased more than 150 arbitrary ELISA units showed significantly improved opsonization in 93% (43/46) of the assays, whereas sera from patients with an increase of antibody concentration of less than 100 arbitrary units showed increased opsonization in only 13% (6/48).

In chemotaxis studies, five of the patient sera were chosen that showed the most enhanced increase of opsonic activity, as measured by killing capacity of pneumococci, and at least a 2-fold increase of antibody concentration. No increased chemotactic activity in postvaccination serum was induced by type 6A, while type 19F induced enhanced chemotaxis in two postvaccination sera and type 23F in one.

Thus, a good correlation between increase of antibody concentration and bacterial killing was found. On the other hand, the antibody response correlated poorly to chemotaxis.

General discussion

Phagocytosis by granulocytes is essential for defence against pneumococci in humans (cf. Densen & Mandell, 1980). Previous in vitro studies on phagocytosis of pneumococci have shown great variation between pneumococcal types (Braconier & Odeberg, 1982). Furthermore, no consistent correlation between the resistance against killing in vitro and the virulence of the type has been found (Braconier & Odeberg, 1982).

In study V, a close correlation was found between enhanced granulocyte killing of pneumococci types 6A, 19F and 23F and increase in antibody concentration against these types after pneumococcal vaccination. Other investigators have not been able to demonstrate a constant relation between rise in specific antibody concentration and enhancement of serum opsonic activity, a discrepancy due perhaps to the opsonic assays used (Giebink et al, 1980; Staehr Johansen & Karup Pedersen, 1982). Another technical explanation might be the dilution of serum used (Giebink et al, 1980; Staehr Johansen & Karup Pedersen, 1982). Moreover, pneumococci type 3, known to be rather resistant to opsonophagocytic killing (Braconier & Odeberg, 1982), was the test organism used in some of the studies (Giebink et al, 1980; Simberkoff et al, 1980; Staehr Johansen & Karup Pedersen, 1982).

Interaction between pneumococci and specific antibodies is known to activate the complement system, resulting in the formation not only of opsonic but also of chemotactic factors. However, in the present study, increased opsonic activity, as measured by enhanced granulocyte killing, was not consistently accompanied by increased chemotactic activity. It might be possible that a more pronounced complement activation was required to promote chemotaxis of granulocytes, than to improve killing of pneumococci - that is to say, the technique used to study chemotaxis was less sensitive than that used to test bacterial killing. The main point, however, is that an increase in specific antibody concentrations against pneumococci types 6A, 19F and 23F - the types most frequently found in recurrent otitis (Austrian et al, 1977) - improved the opsonizing capacity of the sera.

The importance of specific antibodies for defence against pneumococcal infections has been well demonstrated in the pre-antibiotic era when the administration of pneumococcal hyperimmune serum often saved the lives of patients with severe pneumococcal infections (cf. Heffron 1979). Concerning more localized pneumococcal infections, Prellner and co-workers (1984) found that otitis-prone children had lower

amounts of serum antibodies against the pneumococci types 6A and 19F than children who were not otitis-prone. In children with AOM the period required for the elimination of bacteria has been positively correlated to the amount of specific antibody in their middle ear effusions (Sloyer et al, 1976).

Thus, it seems reasonable to try to increase the amounts of specific pneumococcal antibodies in patients susceptible to pneumococcal infection. Vaccination is one possible way of achieving this, and has also been shown to be protective against severe pneumococcal infections in adults (Riley et al, 1977; Smit et al, 1977). In children, Riley and co-workers (1981) reported a reduced number of lower respiratory tract infections caused by vaccine-types, and a number of studies have been undertaken to study the effect of vaccination on pneumococcal AOM in children (Mäkelä et al, 1980; Sloyer et al, 1981; Teele et al, 1981; Wright et al, 1981). The purpose of the present study on pneumococcal vaccination was to evaluate the effect on the overall incidence on AOM - not only pneumococcal AOM - and also the influence on other respiratory tract infections as well as on the nasopharyngeal carriage of potential pathogens. Furthermore, in contrast to all previous studies, even children with no history of AOM were included.

Day-care centres were chosen for the investigation since a homogeneous population of children was required for the study. Furthermore, the children were in daily contact and thereby exposed to the same bacterial strains. Finally, children in day-care centres have previously been shown to be particularly susceptible to upper respiratory tract infection (Hesseltvik, 1949; Ståhlberg, 1980), which has been found to account for approximately 90% of all acute diseases in children in day-care centres (Beem, 1969). Thus, our study ensured similar environmental factors for all the children studied.

The study was designed as a double-blind trial and the children were pair-matched. The matching implied that, for example, otitis-prone children were equally distributed between the vaccine and placebo groups. This design was not used in previous studies.

As to the diagnosis, myringotomy was not regularly performed, but this was not considered a major disadvantage since most of the AOM diagnoses were made by the same two ENT-specialists using otomicroscopy.

Saline with the same preservatives as in Pneumovax[®] was used in control children, in contrast to the other bacterial carbohydrate vaccines that were used as placebos in other investigations (Mäkelä et al, 1980; Sloyer et al, 1981; Teele et al, 1981). The intention was to avoid polyclonal stimulation of antibody producing cells in the controls, which might have been induced by other bacterial vaccines (Coutinho & Möller, 1975; Banck & Forsgren, 1978; cf. Möller, 1979).

The results of the trial showed a reduction of AOM episodes after vaccination in children 2-5 years old when vaccinated. The design of the present study did not allow any conclusions to be drawn as to whether the effect was due to diminished infections by pneumococcal types included in the vaccine, as reported by Mäkelä and co-workers (1980). The possibility that the frequency of AOM caused by other pathogens might increase parallel with a reduction of episodes due to pneumococcal vaccine types was not sustained. Neither H.influenzae, B.catarrhalis or β -haemolytic streptococci were isolated more frequently in vaccinees with AOM than in controls (II).

In children under the age of 2 when vaccinated, no beneficial effect of vaccination on the incidence of AOM was found. Although these children were only given half the dose administered to children 2-5 years old, this dose has been used in young children in recent years (Mäkelä et al, 1980; Sloyer et al, 1981; Douglas et al, 1983), and recent studies in adults have shown as great an increase of serum antibody concentration with 25 μ g as with 50 μ g (Cano et al, 1983). This lack of effect in children under 2 years of age is probably due to the low immunogenicity of polysaccharides in this age group rather than to the reduced strength of the dosage (Borgoño et al, 1978; Cowan et al, 1978; Sell et al, 1981). Serum antibody response to immunization with pneumococcal types frequently isolated in otitis has been found to be poor in infants (Douglas et al, 1983).

In contrast to the findings of the present study, Mäkelä and co-workers (1980) and Teele and colleagues (1981) reported a beneficial effect of the vaccine also in children under 2 years of age, though other investigators have not been able to confirm this (Sloyer et al, 1981; Wright et al, 1981; II). No clinical protection at all was found against AOM caused by type/group 6 - known to be a very poor immunogen - at any age in the study of Mäkelä and co-workers (1980). For other types the protective efficacy was correlated with serum antibody response (Mäkelä et al, 1980).

The reduced rate of tonsillo-pharyngeal infections in the children vaccinated at 2-5 years of age (I) was unexpected, since β -haemolytic streptococci and not pneumococci are known to be a more common cause of throat infection (Glezen et al, 1967; Hovellius et al, 1983). Since the pneumococcal carrier rates were not influenced by vaccination (III), it might be speculated whether antibodies raised to antigens in the vaccine might cross-react with other bacterial antigens or that polyclonal stimulation of lymphocytes (Coutinho & Möller, 1975; Banck & Forsgren, 1978) by the vaccine could be involved. Another possibility is that the reduction of pneumococcal infections demonstrated after vaccination (Mäkelä et al, 1980) leads to diminished trauma to the mucous membrane and thus renders the respiratory tract less susceptible to infections in general. The lack of effect against AOM (II) in vaccinees under 2 years of age - probably due to low immunogenicity of polysaccharides in small children (Borgoño et al,

1978; Cowan et al, 1978; Sell et al, 1981; Douglas et al, 1983) - could at least partly be the reason for the lack of decrease of tonsillitis-pharyngitis episodes in this age group. However, the actual increase of episodes of tonsillitis in children under 2 years of age at vaccination, still calls for an explanation.

The pathogenesis of AOM includes the presence of potentially pathogenic bacteria in the upper respiratory tract, functional or mechanical obstruction of the Eustachian tube and inflammatory and immunological host responses (cf. Giebink & Quie, 1978).

It has been suggested that otitis-prone children are more likely to carry pathogens in the nasopharynx than children less susceptible to otitis (Branefors-Helander et al, 1972; Freijd et al, 1984). In the present study, pneumococci, H.influenzae and B.catarrhalis were found at least as frequently in non-otitis-prone as in asymptomatic otitis-prone children. Acquisition and duration of carriage of pneumococci were markedly similar in the two groups. The isolation frequencies of types/groups 6, 14, 19 and 23 that are common in AOM did not differ between non-otitis-prone and otitis-prone children whether they were studied in an asymptomatic phase or at AOM episodes. These results suggest that the frequency with which different pneumococcal types cause AOM is proportional to the rates of occurrence in the nasopharynx. Also in adherence studies, similarities in adhesive capacity were found between strains isolated from patients with frequent otitis episodes and those from healthy carriers (Andersson et al, 1981). Thus our data did not sustain those of previous studies (Loda et al, 1975; Austrian et al, 1977), where type 14 was found to be carried infrequently but was nevertheless a major cause of AOM (Austrian et al, 1977).

The presence of bacteria in the nasopharynx is generally regarded as a prerequisite for the establishment of AOM, since the middle ear pathogen is frequently recovered from this source (Schwartz et al, 1979). The present study clearly indicated that carriage of bacteria per se did not increase the risk of developing a middle ear infection.

The vaccination did not affect carriership of pneumococci in any age group (III). This finding is not in disagreement with the findings of Herva and colleagues (1980) or Wright and co-workers (1981) who studied one postvaccinational specimen per child. MacLeod and co-workers (1945), however, found reduced carrier rates for some pneumococcal types after vaccination, although this was in adults. Since adults respond better to pneumococcal vaccination than children (Borgoño et al, 1978; Cowan et al, 1978), it might be speculated that an increased serum antibody concentration could be protective against carriage as well as against invasive infection.

As in previous reports, children under 2 years of age carried pneumococci more often than those over 2 years old (Hendley et

al, 1975; Loda et al, 1975). Although crowding at home has been found to be an important factor (Brimblecombe et al, 1958), in the present study the number of family members did not affect carriage, probably due to the fact that exposure to pneumococci was so very much greater in the day-care centres.

Children in day-care centres are frequently carriers of pneumococci (Loda et al, 1975). In the present study a significantly higher carriage rate was found among those children attending more crowded day-care centres.

The finding that types/groups 6, 14, 19 and 23 were those most frequently acquired and carried for long periods is in agreement with previous reports (Masters et al, 1958; Gray et al, 1980). These types/groups are also known to be responsible for more than half of all pneumococcal AOM episodes (Austrian et al, 1977).

Loda and colleagues (1975) found a limited spread of pneumococcal types, frequently found in AOM, among children in a day-care centre in spite of prolonged contact. However, in the present investigation a prompt but brief spread was noted.

The present investigation shows that carriage of pathogens in the nasopharynx per se does not increase the risk of developing AOM (IV). Since the relationship between carriage and the development of infection is unclear, no conclusions can be drawn as to whether it is better, from that point of view, to have large day-care centres with their increased carrier rates of potential pathogens than small ones with their lower rates (III).

Pneumococcal vaccination did offer some protection against upper respiratory tract infections such as AOM, pharyngitis and tonsillitis, with minimal side effects in children over 2 years of age. Attempts to protect younger children by vaccination who are highly susceptible to AOM (Howie, 1975) have, however, failed (Sloyer et al, 1981; Wright et al, 1981; I, II).

In conclusion, while a general immunization of children with the pneumococcal vaccine available at present cannot be justified, vaccination might be recommended as a supplement to the usual AOM treatment to prevent recurrent attacks of AOM in selected children between the ages of 2 and 5.

General summary

Children, aged 6 months to 5 years and attending day-care centres, were prospectively studied with regard to bacterial carriage and upper respiratory tract infections, especially acute otitis media (AOM). The effect of pneumococcal vaccination on these infections, on bacterial carriage and on opsonization of pneumococci was evaluated.

Nasopharyngeal cultures were taken monthly and pneumococci were frequently (31.9%) isolated from the nasopharynx in the children, especially in those under 2 years of age. Pneumococci were recovered from more than 50% of the cultures in 16% of the children. The most common types/groups isolated were 6, 23, 19 and 14. From one child 7 different types/groups of pneumococci were isolated during the 2-year study period whereas no isolation of pneumococci at all was registered in 19 children. Pneumococci of group 6 were those carried most frequently and for longer periods than other types/groups. In day-care centres attended by 45 children or more the carriage rate of pneumococci was significantly higher than in centres with less than 45 children. Spreading within centres was common but of short duration.

Carriership of pneumococci, H.influenzae and B.catarrhalis in the nasopharynx was at least as common in non-otitis-prone children as in asymptomatic otitis-prone children. In 3-4 year-old children B.catarrhalis were isolated more often in non-otitis-prone children than in asymptomatic otitis-prone children. Thus, the presence of potential pathogens per se did not seem to increase the risk of developing AOM. The frequency with which different pneumococcal types/groups were present in AOM was proportional to the rates of carriage in asymptomatic children. These data emphasize the importance of host factors in the development of AOM and do not support the existence of specific "otitis-strains" among the bacteria harboured in the nasopharynx.

In in vitro studies, enhanced bacterial killing of the pneumococcal types 6A, 19F and 23F, commonly encountered in AOM, was found to parallel increased concentrations of antibodies achieved after pneumococcal vaccination.

Pneumococcal vaccination reduced the number of repeated episodes of AOM in children between the ages of 2 and 5 when vaccinated. The duration of protective efficacy was demonstrable for at least one year. In children 2 to 5 years old a reduced frequency of tonsillitis and pharyngitis was also seen after vaccination. No protective effect of vaccina-

tion was seen in children vaccinated before 2 years of age. Pneumococcal vaccination did not affect nasopharyngeal carriership of pneumococci. No severe adverse reaction was seen after vaccination. Thus, vaccination might be recommended as a supplement to the usual AOM treatment, to prevent recurrent attacks of AOM in children between the ages of 2 and 5.

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Paper I

Effect of Pneumococcal Vaccination on Upper Respiratory Tract Infections in Children

Design of a Follow-up Study

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ABSTRACT. Children, aged 6 months to 5 years, were pair-matched with respect to age and history of otitis media acuta. The 405 children received either a subcutaneous injection of Pneumovax® (a 14-valent pneumococcal vaccine) or of saline, in a double-blind fashion. The follow-up period for each child was 1½ years. In children younger than 2 years at vaccination no difference was recorded between vaccinees and controls concerning the number of consultations due to otitis media acuta or to other upper respiratory tract infections. In children older than 2 years at vaccination a reduction in the overall frequency of visits due to upper respiratory tract infections was observed after vaccination. The incidence of otitis media acuta was reduced during the first postvaccinal year while visits due to tonsillitis and pharyngitis were reduced during the whole follow-up period in vaccinees as compared to controls.

INTRODUCTION

It is well established that vaccination against pneumococci prevents septicemia and pneumonia with these bacteria in adults (1, 3, 22). Reduced mortality in lower respiratory tract infections as well as reduced frequency of recurrent pneumococcal otitis media acuta has been demonstrated in children following vaccination (18, 20, 21, 25). The majority of otitis media acuta cases are caused by pneumococcal types included in the currently available vaccines (4, 14). The effect of pneumococcal vaccination on other respiratory tract infections is not known and the role of pneumococci in respiratory tract infections other than otitis media acuta has received little attention. A main obstacle in establishing a pneumococcal etiology in e.g. rhinitis or pharyngitis is the high carrier frequency among children of potentially pathogenic bacteria, e.g. *H. influenzae*, *B. catarrhalis* and pneumococci (8, 15, 17). Since these infections constitute a major quantitative problem in general practice it was of interest to study the effect of pneumococcal vaccination on upper respiratory tract infections of all types. The present report concerns vaccination of children attending day-care centers. The study was designed as a double-blind trial in children aged 6 months to 5 years comparing the effect of Pneumovax® and

placebo injections on the overall incidence of upper respiratory tract infections. The present evaluation was performed 1½ years after the immunization.

DESIGN OF THE STUDY

The children included in the study were recruited from 66 day-care centers in the municipalities of Lund attended by approximately 4000 children. Information meetings were held at the day care centers. The parents were given oral information and a written description of the project and were encouraged to decide about their children's participation after considering the information "for some days". They were then invited to send a written consent if they agreed to participate. The design of the study was approved by the ethical committee at the University of Lund.

Information concerning the medical history of the child was obtained on a questionnaire. Specific questions were asked concerning pregnancy and delivery, congenital malformations, chronic diseases, allergy, recurrent upper respiratory tract infections including otitis media acuta and tubulation of the ear, common infant virus maladies and other previous infections. Information was also obtained concerning the number and age of family members and the housing conditions.

Date _____

Patients name _____ Birthday _____

History

Day of falling ill _____ Cough ☐ Respiratory distress ☐
 Fever ☐ Rhinitis ☐ Ear discharge ☐
 Irritable ☐ Throat pain ☐
 Refuse to eat ☐ Ear pain ☐

Other comments _____

Status

General condition: normal ☐ comments _____
 Ear, right : normal ☐ comments _____
 Ear, left : normal ☐ comments _____
 Mouth, throat : normal ☐ comments _____
 Nose : normal ☐ comments _____
 Epiqlottis : normal ☐ comments _____
 Pulm : normal ☐ comments _____
 Neck, l.nodes : normal ☐ comments _____
 Other : _____

Bacteriol. specimens from nasoph ☐ throat ☐ ear ☐ conj ☐ other _____Treatment: PcV ☐ ampi ☐ ery ☐ doxy ☐ trim-sulf ☐ dose _____antihist ☐ rhin.gut. ☐ expect ☐ Drug _____ dose _____Diagnosis: acute otitis ☐ acute rhinitis ☐ acute tonsill. ☐acute pharyngitis ☐ acute bronchitis ☐ acute laryngitis ☐

other _____

Bacteriological examinations, results (to be filled in later)

Fig. 1. The formula used for registration in acute visits.

Children with chronic or progressive diseases or known immunological disorders were excluded from the study as were children with cleft palate.

The study was designed as a double-blind matched study in children 6 months to 5 years of age to determine the effect of a 14-valent pneumococcal polysaccharide vaccine (Pneumovax®, supplied by Merck, Sharp & Dohme, West Point, Pennsylvania, USA) on upper respiratory tract infections. The vaccine contained capsular type antigens from types 1, 2, 3, 4, 6A, 7F, 8, 9N, 12F, 14,

18C, 19F, 23F and 25 (Danish nomenclature) and was administered subcutaneously in the lateral upper part of the arm. The children were matched in pairs with respect to age and absence/presence of previous attacks of otitis media acuta. One child in every pair of children, older than 2 years, received 0.5 ml vaccine (50 µg of each pneumococcal polysaccharide) and the other saline containing preservatives. Infants younger than 2 years received 0.2 ml (25 µg of each pneumococcal polysaccharide).

Every week-day during the follow-up period the

Table I. Age and sex distribution of controls and vaccinees

Group	Status	Number	Mean age (years)	Age range	Per cent male
1	C	22	1.46	0.89-1.92	55
	V	18	1.38	0.67-1.87	56
2	C	26	1.50	0.74-1.97	73
	V	26	1.50	0.73-1.90	54
3	C	47	3.82	2.07-5.85	40
	V	47	3.67	2.11-5.85	51
4	C	112	3.81	2.00-5.76	54
	V	107	3.77	2.04-5.98	58
Total	C	207	3.27	0.74-5.85	54
	V	198	3.23	0.67-5.98	55

staff at the day-care centers recorded symptoms of respiratory tract infections. The parents also made daily registrations concerning nasal discharge, cough, tiredness and fever. These registration formulas were sent monthly to the investigators. Cultures from the nasopharynx and the throat were taken from all the children once a month by a trained nurse.

When the children needed medical care they were requested to attend a special reception which was opened at the ENT Clinic at the University Hospital in Lund. Two of the investigators who were ENT specialists were available at the reception one hour daily on week-days. Children requesting medical help on other hours were investigated and treated by the ENT physician on duty. About 20% of the children chose to visit general practitioners. At each visit a special formula for registration of disease history, objective findings, diagnosis and therapy was used in addition to the usual medical record (Fig. 1).

Throat and nasopharyngeal specimens were taken at every visit and middle ear effusion was aspirated when myringotomy was performed. The therapy given at consultations followed general recommendations.

The specimens were examined for: pneumococci, which were typed by Quellung reaction (19); β -hemolytic streptococci, which were serologically grouped and T-typed (6, 11); *H. influenzae* strains, which were serologically typed and biotyped (13); and *Branhamella catarrhalis* (12). The *H. influenzae* and *B. catarrhalis* strains were tested for β -lactamase production.

STUDY POPULATION

In all 405 children were included. They were allocated according to age and previous history of otitis media acuta to one of the following groups:

1. <2 years of age with no previous episode of otitis (40 children)
2. <2 years of age with previous episode(s) of otitis (52 children)
3. >2 years of age with no previous episode of otitis (94 children)
4. >2 years of age with previous episode(s) of otitis (219 children).

In all 198 children were vaccinated and 207 served as controls.

The demographic data for vaccinees and controls are given in Table I. Although the proportion of girls in group 2 seemed to be higher among the vaccinees there was no significant difference between vaccinated children and controls as to age and sex (Table I). Furthermore, there was no difference between the groups concerning medical history such as allergy, tubulation of ear drums or other data according to the questionnaire.

Side-effects to vaccination/placebo injection were registered by the parents on a questionnaire and were reported to 31% in the vaccinees and to 1.5% in the controls. Temperature above 38°C was reported by 3% of the vaccinees whereas the majority of complaints registered concerned local erythema or swelling. No severe reactions were registered, which was in accordance with previous reports (5, 26).

RESULTS OF THE 1½ YEAR FOLLOW-UP

During the 1½ year follow-up 517 visits due to upper respiratory tract infections in 228 children were registered.

In children younger than 2 years at vaccination the number of visits due to upper respiratory tract infections (including otitis media acuta) was the same among vaccinees as among controls, 114 and 109, respectively. The number of recognized episodes of acute otitis media was also the same in the two groups, 48 and 41.

In children 2 to 5 years old at vaccination a significantly lower number of visits due to upper respiratory tract infections was recorded, 124 among vaccinees and 170 among controls ($p < 0.01$) (Table II).

Table II. Number of visits for all respiratory tract infections among 154 vaccinees and 159 controls older than 2 years when vaccinated

Episode	Vaccinees	Controls
First	71	83
Second	26	41
Third	14	23
Fourth	6	12
Fifth	3	5
Sixth or more	4	6
Total	124	170

As compared with the controls the number of visits over the periods 0–6, 7–12 and 13–18 months after vaccination was decreased by 20%, 30% and 27%, respectively. However, the number of upper respiratory tract infections was not statistically significant between vaccinees and controls for any of the periods taken alone.

Over the first postvaccinational year the incidence of otitis media acuta among children, with or without previous otitis media, was significantly reduced among vaccinees as compared to controls, 39 and 58 episodes, respectively ($p < 0.05$). Over the whole follow-up period the corresponding figures were 52 and 69 (Table III).

The number of visits due to upper respiratory tract infections other than otitis media acuta was also lower among vaccinees than among controls. For tonsillitis and pharyngitis a statistically significant difference was recorded ($p < 0.05$) (Table IV).

There was no difference in the frequency of isolations of pneumococci, *H. influenzae*, *B. catarrhalis*

Table III. Number of acute otitis media (AOM) episodes among 154 vaccinees (V) and 159 controls (C) older than 2 years when vaccinated

Episode	Children with no previous history of AOM		Children with previous history of AOM	
	V	C	V	C
First	4	7	32	34
Second		1	10	12
Third		1	4	7
Fourth or more			2	7
Total	4	9	48	60

Table IV. Number of visits for some respiratory tract infections other than otitis media in 154 vaccinees and 159 controls older than 2 years when vaccinated

Diagnosis	Vaccinees	Controls
Rhinitis	58	65
Pharyngitis	10	29
Tonsillitis	10	24

or β -hemolytic streptococci when comparing vaccinated children and controls (Table V). Pneumococcal types included in the vaccine were found in 12 episodes of otitis media acuta in vaccinated children older than 2 years compared to 19 in the controls. In 26 otitis episodes no pneumococci were isolated in vaccinated children older than 2 years, whereas the corresponding figures for the controls were 37 episodes.

DISCUSSION

Children attending day-care centers were chosen for this study for several reasons. They constitute a homogeneous population where vaccinees and controls mix daily and are therefore exposed to the same microbial agents. Several investigators have reported a higher frequency of respiratory tract infections among children attending day-care centers than in those cared for at home or in family day-care homes (9, 24). Furthermore, small children cared for outside their home, are more prone to acquire otitis media acuta (10, 23).

The present trial estimated the effect of vaccination not only on otitis media acuta but also on other respiratory tract infections, irrespective of etiological agents. During the 1½-year follow-up a reduced number of visits due to upper respiratory tract infections in children vaccinated at the age of 2–5 years was recorded. Unexpectedly, the effect of vaccination was most pronounced on pharyngitis and tonsillitis, infections in which the role of pneumococci is practically unknown (16). The reduction in upper respiratory tract infections was accompanied by a diminished number of isolates, not only of pneumococcal vaccine types but also of β -streptococci, *B. catarrhalis* and *H. influenzae*. These find-

Table V. Frequency and number (n) of bacterial isolates for all visits in vaccinees (V) and controls (C) older than 2 years when vaccinated

The number of visits were 124 for vaccinees as compared to 170 for controls

Organism	Isolation frequency (n)	
	V	C
<i>B. catarrhalis</i>	37% (46)	39% (66)
<i>H. influenzae</i>	34% (42)	39% (66)
β -streptococci	15% (15)	18% (30)
<i>S. pneumoniae</i>		
Vaccine-types	23% (28)	25% (42)
Non-vaccine types	8% (10)	4% (7)
Strains not typed	6% (8)	5% (9)
Total	(149)	(220)

ings call for further studies of bacterial upper respiratory tract infections and the possible interference between potentially pathogenic bacteria. Mäkelä and colleagues (18) in their study of the efficacy of pneumococcal vaccine in otitis media acuta registered a reduction in the number of infections with pneumococcal types other than those included in the vaccine. One may speculate that a reduction of pneumococcal upper respiratory tract infections leads to a reduction in trauma to the mucous membranes and thus render the respiratory tract less susceptible to infections in general.

The reduction of the total incidence of otitis media acuta in children above 2 years of age was partly due to a reduction of episodes due to pneumococcal types included in the vaccine which is in accordance with the findings of other investigators (18, 21, 25). One important finding in our study was that otitis media acuta caused by other pathogens did not increase parallelly to the reduction of episodes caused by pneumococci.

Previous studies have shown that children younger than 2 years respond poorly to bacterial polysaccharide vaccine, including pneumococcal antigens (7) possibly because of "immaturity" of the immunological system (2). Thus it came as no surprise that we were unable to demonstrate protection after vaccination against respiratory tract infections in this age group. However, it cannot be excluded that the poor results obtained in these children might have been influenced by the lower dose of vaccine used. However, a dose of 25 μ g of each pneumococcal antigen does not seem to pro-

duce an antibody response significantly different from that of a 50 μ g dose (7). A higher dose might induce tolerance or increase the adverse reactions in small children.

In conclusion, the present study has shown a beneficial effect of pneumococcal vaccination on the incidence of upper respiratory tract infections in children older than 2 years.

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Paper II



BENEFICIAL EFFECT OF PNEUMOCOCCAL VACCINATION ON OTITIS MEDIA
IN CHILDREN OVER TWO YEARS OLD

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SUMMARY

The effect of immunization with a 14-valent pneumococcal vaccine (Pneumovax®) was studied in a double-blind trial in which 405 children between the ages of six months and five years were matched in vaccine/control pairs according to age and history of otitis media. The fact that all the children attended day-care centres ensured that both vaccinees and controls were similarly exposed to upper respiratory tract pathogens. The total incidence of acute otitis media was reduced during a two-year follow-up period by 24% ($p < 0.05$) among those vaccinated between the ages of two and five. For recurrent episodes a decrease by 40% was noted. Protective efficacy was demonstrable during the first post-vaccinational year, but not when more than one year had lapsed after the vaccination. These findings suggest that, while the vaccine had no effect on children under two years old, it may be a useful aid in preventing recurrent attacks of otitis media in children between two and five years of age.

INTRODUCTION

Despite antimicrobial treatment, otitis media remains a major problem in early childhood (1). Different bacteriological studies of middle ear effusions in otitis media have largely agreed as to the frequency of certain pathogens, Streptococcus pneumoniae having been the predominant one (2). Since immunization with pneumococcal vaccine is without serious side effects and has been successful in systemic pneumococcal infections (3, 4, 5), trials of the effect on otitis media have been considered important.

The beneficial effect of pneumococcal vaccination in children below the age of two years (6, 7) has not been confirmed by other investigators (8, 9). In one vaccination trial (6), children above two years of age were included, and a reduction of recurrent acute otitis media (AOM) episodes due to pneumococcal vaccine-types was noted during the six months following immunization.

In three of the above mentioned investigations (6, 7, 9) the control children were vaccinated with other bacterial vaccines whereby a possible polyclonal stimulation of antibody producing cells could have influenced the results. Only children who experienced at least one previous episode of AOM were recruited in these trials. In the study by Wright and co-workers (8) also children without former AOM attacks were included. Their control children did not receive any kind of injection and consequently the trial cannot be considered double-blind.

Environmental factors, known to be important for the rate of upper respiratory tract infections in children, have not been taken into account in the previous studies.

To minimize the drawbacks described above the present study was designed as a double-blind prospective study comparing the effect of a 14-valent pneumococcal vaccine, Pneumovax®, with that of saline, in children under five years old. All the children attended day-care centres and were thus expected to be similarly exposed to upper respiratory tract pathogens. Both children with and without previous AOM were included and the children were pair-matched with regard to this and to their age.

The aim of the present study was to evaluate the effect of pneumococcal vaccination on the overall incidence of AOM in children attending day-care centres.

MATERIALS AND METHODS

Design of the study

The study was designed as a double-blind pair-matched examination of the effect of the administration of a 14-valent pneumococcal vaccine, Pneumovax[®], to children between the ages of six months and five years, on upper respiratory tract infections, including acute otitis media (AOM). The vaccine contained capsular type antigens 1, 2, 3, 4, 6A, 7F, 8, 9N, 12F, 14, 18C, 19F, 23F, 25, and saline was used as placebo. Both vaccine and placebo contained phenol 0.25% as preservative.

The subjects included in the study were recruited among the approximately 4,000 children attending 66 day-care centres in the municipalities of Lund. Parents were given both verbal and written descriptions of the project, and were invited to send written consent if they agreed to let their children participate. The design of the study, including the written information, was approved by the Ethics Committee of the University of Lund.

Allocation groups

Information concerning the children's medical history and present state of health was obtained from a questionnaire answered by their parents. Children with chronic or progressive diseases, or known immunological disorders, were excluded from the study, as were children with cleft palates.

In all, 405 children were included in the study, being matched in pairs within one of the following groups:

1. <2 years of age with no previous episode of otitis (40 children).
2. <2 years of age with previous episode(s) of otitis (52 children).
3. 2-5 years of age with no previous episode of otitis (94 children).
4. 2-5 years of age with previous episode(s) of otitis (219 children).

One child in every pair received vaccine and the other saline. In all, 198 children were vaccinated and 207 served as controls. In Table I the demographic data of the groups are given for both the vaccinees and controls. There were no significant differences between vaccinated children and controls as to age and sex. Furthermore, there was no difference between vaccinees and controls with regard to medical history such as mother's pregnancy and delivery, congenital malformation, allergy, recurrent upper respiratory tract infection, tubulation of the ear, common infant virus maladies and other previous infections. Both number and age

Table I. Age and sex distribution by group for controls (C) and vaccinees (V).

Group	Status	No. of infants	Mean age in (years) range	Per cent females
1	C	22	1.46 (0.89-1.92)	45
	V	18	1.38 (0.67-1.87)	44
2	C	26	1.50 (0.74-1.97)	27
	V	26	1.50 (0.73-1.90)	46
3	C	47	3.82 (2.07-5.85)	60
	V	47	3.67 (2.11-5.85)	49
4	C	112	3.81 (2.00-5.76)	46
	V	107	3.77 (2.04-5.98)	42
Total	C	207	3.27 (0.74-5.85)	46
	V	198	3.23 (0.67-5.98)	45

of family members, as well as housing conditions were similar in both vaccinees and controls.

Vaccination

In those over two years old, one child in each pair received 0.5 ml vaccine (50 µg of each pneumococcal antigen) and the other 0.5 ml saline, while those under two were given 0.25 ml of vaccine or saline. Like in previous studies (3, 6) no serious side effects were noted.

Clinical follow-up

Any child needing medical care during the 2-year follow-up period was asked to attend a special clinic run by two of the authors who were ENT specialists and available for consultation one hour daily on weekdays. Children seeking medical attention at other times were examined and treated when necessary by the duty doctor at the regular ENT clinic at the University Hospital of Lund. About 10% of the children with AOM instead consulted their own general practitioners. At each consultation (whether with ENT specialists or general practitioners) the usual medical record was supplemented by a special form reporting disease history, objective findings, diagnosis and therapy. Any therapy given at such consultations followed normal practice.

Acute otitis media was defined as a wholly red, dull and sometimes bulging ear-drum in a child with signs of upper respiratory tract disease, occasionally febrile, complaining of earache. An otomicroscope was used in all examinations at the ENT clinic.

Bacteriological specimens

Pneumococci, H.influenzae and β -haemolytic streptococci were identified by conventional methods, and pneumococcal types were determined by Quellung reaction (10, 11). H.influenzae strains were serologically typed. B.catarrhalis was identified as described by Kamme (12).

Statistical analysis

The χ^2 test was used for statistical analysis.

RESULTS

Children <2 years of age at recruitment

Forty-one of the 92 children less than two years old when recruited accounted for 123 episodes of otitis media during the two-year follow-up period. Episodes were as frequent among the 44 vaccinees (67 episodes) as among the 48 controls (56 episodes) ($p > 0.05$). Nor was there any difference in the number of otitis media episodes between vaccinees and controls aged one to two years at recruitment. Within groups 1 and 2, no differences were seen between vaccinees and controls.

Children 2-5 years of age at recruitment

101 of the 313 children, two to five years old when vaccinated or given saline, accounted for 177 episodes of otitis media (Table II). Seventy-five of these cases occurred in vaccinees and 102 in the controls ($p < 0.05$), a decrease per child of 24% in the vaccinees.

Among children experiencing their first episode, there was no numerical difference between the vaccination group and the control group, (47 and 54 episodes, respectively). The number of subsequent episodes, however, differed significantly (28 and 48 episodes respectively; $p < 0.05$), representing a decrease of 40% in the vaccinees.

Table II. *Number of acute otitis media episodes among 154 vaccinees and 159 controls, 2-5 years of age at recruitment for the study.*

Episode	Vaccinees (n = 154)	Controls (n = 159)
First	47	54
Second	17	26
Third	6	11
Fourth	3	3
Fifth	1	3
Sixth	1	2
Seventh		2
Eighth		1
Total No. of episodes	75	102

$p < 0.05$, χ^2 test

Within the group with no previous history of otitis media at recruitment, 7 episodes occurred in 47 vaccinated children and 15 in 47 controls ($p>0.05$). In children with previous history of otitis media 68 episodes occurred in 107 vaccinees, as compared with 87 in 112 controls ($p<0.05$).

The incidence of otitis media related to time of occurrence is shown in Table III, a 30% reduction being shown in vaccinees during the first year after vaccination ($p<0.05$), but no such effect during the second year.

Isolation of bacteria from the nasopharynx of children with acute otitis media

The number of pathogens in nasopharyngeal cultures taken at medical consultations due to acute otitis media in vaccinees and controls over two years old when vaccinated is shown in Table IV.

Types included in the vaccine were isolated in 10 (AOM) episodes among vaccinees as compared with 17 among controls ($p>0.05$). The corresponding data for non-vaccine types were 22 episodes among vaccinees and 17 among controls ($p>0.05$).

There was no difference between vaccinees and controls with regard to the number of instances where H.influenzae, B.catarrhalis or β -haemolytic streptococci were isolated. 86% of H.influenzae strains were non-capsulated.

Table III. *Number of acute otitis media episodes among 154 vaccinees and 159 controls, 2-5 years of age at recruitment for the study: Relation to time of occurrence after recruitment.*

Time after recruitment	Vaccinees (n = 154)	Controls (n = 159)
0 - 1 year*	47	69
> 1 year**	28	33
Total	75	102

* $p < 0.05$, χ^2 test

** N.S. " "

Table IV. *Number of isolations of pneumococci, H.influenzae, B.catarrhalis and β -haemolytic streptococci from the nasopharynx in 177 AOM episodes among vaccinees (n = 154) and controls (n = 159) aged 2-5 years.*

Bacteria	Number of isolates with respective bacteria	
	vaccinees	controls
Pneumococci		
vaccine types	10	17
non-vaccine types	22	17
H.influenzae	28	26
B.catarrhalis	30	33
β -haemolytic streptococci	1	2

DISCUSSION

This study was designed as a pair-matched vaccine/placebo trial in children attending day-care centres. The environment ensured similar exposure of both vaccinees and controls to upper respiratory tract pathogens, while the matching ensured that otitis-prone children were equally distributed between the vaccine and the placebo groups. The milieu aspects have been insufficiently taken into consideration in previous investigations. Finally, in contrast to the procedures in other investigations, we used saline as the placebo, thereby excluding the possibility of unspecific polyclonal antibody stimulation (13, 14). In our study myringotomy was not performed regularly in children with otitis media, as it was, for example, in the study of Mäkelä and coworkers (6) and this might represent a diagnostic drawback, which, however, was counteracted by the fact that up to 85% of the diagnoses were made by the same two ENT specialists using otomicroscopy.

The reduction of the total incidence of AOM after pneumococcal vaccination is of crucial importance in clinical practice. During the two-year follow-up, there was a 24% total reduction of otitis media episodes in children two to five years old when vaccinated. The reduction was due to fewer second and subsequent attacks after vaccination, and not to fewer first episodes. Mäkelä and co-workers (6) found, in children more than two years old, a reduction of AOM episodes due to pneumococcal types included in the vaccine. A correlation exists between the middle ear pathogen and the bacteria present in nasopharynx (15, 16, 17, 18). Our data from nasopharyngeal isolates are not inconsistent with the findings of Mäkelä and colleagues (6). The possibility that the frequency of AOM caused by other pathogens might increase parallelly to the reduction of episodes due to pneumococcal vaccine-types was not sustained. None of the pathogens were isolated more frequently in vaccinees with AOM than in controls. The duration of protective efficacy was in contrast to the findings in a previous study (6), demonstrable throughout the entire first year after vaccination. The results clearly indicated that children over two years old may benefit by pneumococcal vaccination. However, no effect was obtained by vaccination of younger infants. This is in accordance with other investigators who vaccinated children between the ages of 6 and 12 months (8) and 6 and 21 months (9), respectively, and found otitis media to occur with equal frequency in vaccinated children and controls. The lack of beneficial effect of vaccination in children below the age of two years is consistent with the low immunogenicity of polysaccharide vaccines in this age group (19, 20).

We immunized children younger than two years with a dose half that of older children, 25 µg of each pneumococcal antigen, in order to avoid induction of tolerance or adverse reactions. This dose has been currently in use (6) and does not seem to

produce an antibody antigen response significantly different from that of a 50 μ g dose (19, 20, 21).

In conclusion, vaccination with Pneumovax[®] reduced the number of AOM attacks in children between the ages of two and five. The reduced frequency of such infections after two years of age does not justify the general use of the vaccine for otitis in this age group. However, based on the present study and previous observations (6), vaccination may be indicated to prevent recurrent attacks of AOM in children between the ages of two and five.

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Paper III

A LONGITUDINAL STUDY OF THE NASOPHARYNGEAL CARRIAGE OF
PNEUMOCOCCI AS RELATED TO PNEUMOCOCCAL VACCINATION IN
CHILDREN ATTENDING DAY-CARE CENTRES

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ABSTRACT

A long-term study of nasopharyngeal carriage in 405 children, aged 6 months to 5 years, attending day-care centres, was performed. The effect of pneumococcal vaccination was evaluated in a double-blind investigation where the children received either Pneumovax® (a 14-valent pneumococcal vaccine) or saline. Nasopharyngeal cultures were taken monthly by a trained nurse during a 2-year follow-up period. No difference in pneumococcal carriage was found between vaccinees and controls. Pneumococci were found in 31.9% of all cultures. In day-care centres attended by ≥ 45 children the carriage rate of pneumococci was significantly higher than in centres with less than 45 children. Spreading of pneumococci within day-care centres was common but rather short-lived. Children younger than 2 years showed the highest carriage rates. Pneumococci of group 6 were carried most frequently and for longer periods than other types/groups.

INTRODUCTION

A great number of healthy individuals are carriers of pneumococci in the upper respiratory tract. The rate of asymptomatic carriage is highest in children of pre-school age and tends to decrease with increasing age (1, 2, 3, 4). Although common colds seem to promote nasopharyngeal acquisition of bacteria (5, 6, 7), the mechanisms by which pneumococci colonize the upper respiratory tract and occasionally become invasive are not fully understood.

The pneumococcal types most frequently isolated in healthy carriers are also those commonly associated with pneumococcal upper respiratory tract infections (8, 9, 10, 11).

Attempts to eliminate pneumococci from the nasopharynx with penicillin have only temporarily been successful (11, 12).

We have previously reported on the beneficial effect of pneumococcal vaccination on the overall frequency of upper respiratory tract infections including otitis media acuta in children above 2 years of age (13). The aim of the present study was to investigate the epidemiology of pneumococcal carriage in children attending day-care centres and to evaluate the possible influence of pneumococcal vaccination on carriership.

MATERIALS AND METHODS

Design of the study

The study was performed as a double-blind, pair-matched investigation of children between the ages of six months and five years, to determine the effect of immunization with a 14-valent pneumococcal vaccine, Pneumovax®, on nasopharyngeal carriage of pneumococci. The vaccine contained capsular type antigens 1, 2, 3, 4, 6A, 7F, 8, 9N, 12F, 14, 18C, 19F, 23F, and 25. Saline was used as placebo. Both vaccine and placebo contained phenol 0.25% as preservative.

It was planned to recruit 400 children with at least 100 of them younger than 2 years of age. To obtain this, the parents of 4,000 children attending 66 day-care centres in the municipalities of Lund were given both verbal and written descriptions of the project, and were invited to send written consent if they agreed to let their children participate. Information concerning the children's medical history and social data was obtained from a questionnaire answered by their parents. The design of the study, including the written information, was approved by the Ethics Committee of the University of Lund.

Allocation groups

In all, 405 children were included in the study, and were matched in pairs within one of the following groups:

1. <2 years of age with no previous episode of otitis media acuta (40 children)
2. <2 years of age with previous episode(s) of otitis media acuta (52 children)
3. 2-5 years of age with no previous episode of otitis media acuta (94 children)
4. 2-5 years of age with previous episode(s) of otitis media acuta (219 children)

One child in every pair received vaccine and the other saline. In all, 198 children were vaccinated and 207 served as controls. There was no difference between vaccinees and controls with regard to medical history except otitis. Number and age of family members, as well as housing conditions were similar for both vaccinees and controls.

Vaccination and follow-up

For those over 2 years of age, one child in each pair received 0.5 ml vaccine (50 µg of each pneumococcal antigen) and the other 0.5 ml saline, while those under 2 years of age were given 0.25 ml of vaccine or saline. As reported previously no serious side effects were noted (13). The children were observed for 2 years after vaccination.

Each day-care centre was visited once a month by the same trained nurse during the follow-up, with the exception of July and August (the larger majority of infants were absent from the centres during this time). Specimens were then collected with a cotton wire swab from the nasopharynx of children enrolled in the study, provided they were not taking antibiotics. During the 2-year follow-up, 3,418 specimens from the 405 children were cultured, an average of 8.4 per child. The absence of the children at the monthly visits was mainly due to illness or holidays.

Bacteriological specimens

The nasopharyngeal specimens were investigated for pneumococci. Pneumococci were identified by conventional methods and types were determined by the Quellung reaction (14, 15).

Statistical analysis

The χ^2 test was used for statistical analysis.

RESULTS

I. Effect of pneumococcal vaccination on nasopharyngeal carriage

Totally, pneumococci were isolated from 1,091 of the 3,418 routine cultures, 31.9%. The nasopharyngeal carriage rate of pneumococci was similar for vaccinees (538 isolates, 31.7%) and controls (553 isolates, 32.1%).

Children younger than 2 years at the time of culture carried pneumococci more often than older children, 46.2 versus 30.6%, respectively ($p < 0.001$). The rate of pneumococcal carriage did not differ among the children depending on history of acute otitis media. No differences were seen in pneumococcal carriage between vaccinees and controls, whether above or below 2 years at time of vaccination.

The number of members in the child's family did not affect carriage rates. Thus, the carriage rates in children living in families with 2 members including the child, were 37.0%. The corresponding data for children in families with 5 members or more were 36.8%.

Table I. *Isolation frequency of pneumococcal types/groups from vaccinees and controls at routine cultures. (Types/groups with < 20 isolates are grouped together.) Types/groups included in the vaccine are indicated by (V) after type/group.*

Number of positive cultures among				
Type/group	Vaccinees	Controls	Total	(per cent of pneumococcal isolations)
3 (V)	9	16	25	(2.3)
6 (V)	131	124	255	(23.4)
11	32	28	60	(5.5)
14 (V)	37	48	85	(7.8)
15	23	33	56	(5.1)
18 (V)	26	27	53	(4.9)
19 (V)	74	73	147	(13.5)
23 (V)	74	87	161	(14.8)
35	30	24	54	(4.9)
Other types or non-typable	102	93	195	(17.9)
Total pneumococci	538	553	1,091	

Percent
pneumococci

-7-

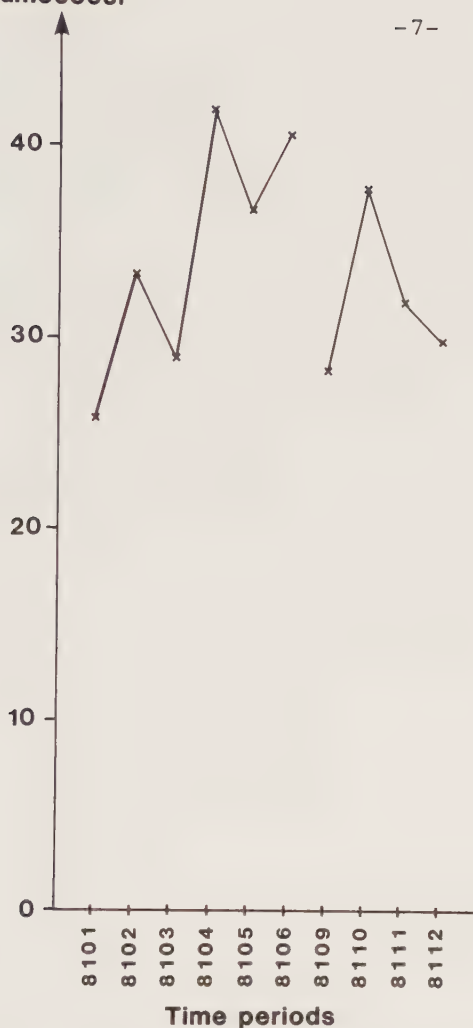


Fig. 1. Rate of pneumococcal carriage measured in monthly periods during 1981 (July-August 1981 excluded).

During examination of pneumococcal carriage in vaccinees and controls month by month during the 2-year follow-up the proportion of children colonized did not differ between vaccinees and controls for any of the periods. Data from 1981 are shown in Fig. 1. The highest proportion of children harbouring pneumococci was found in April-June (around 40%) and October (around 35%) whereas September and December-January showed less colonized infants (below 30%). Carriage rates for January and September were significantly lower than for April and October ($p < 0.05$).

As can be seen in Table I there was no difference in isolation frequency between vaccinees and controls for pneumococci of vaccine types/groups. Also when pneumococcal isolates of group 6, known as a very poor immunogen in children (16), were excluded, the nasopharyngeal carriage was similar in controls and vaccinees for the other vaccine types/groups.

II. Acquisition, reacquisition and length of carriage

Since no effect of vaccination on pneumococcal carriage could be demonstrated the following results refer to combined data for vaccinees and controls.

In order to study the spreading of pneumococci in the day-care centres the children who had had specimens taken at 10 times or more with an interval of less than 45 days between two samples were selected and thoroughly examined. One hundred and sixty children fell into this category. From this selected group of children, 1,811 specimens were taken.

The individual frequency of cultures positive for pneumococci in these children is shown in Table II. Pneumococci were recovered from more than 50% of the cultures in 26 (16.3%) of the children, whereas 41 (25.6%) harboured pneumococci in less than 10% of their cultures.

Table II. *Frequency of pneumococcal carriage in 160 children from whom ten or more routine cultures were obtained.*

Per cent of all cultures from each child positive for pneumococci	n (%)
0	19 (11.9)
1-9	22 (13.8)
10-19	28 (17.5)
20-29	21 (13.1)
30-39	17 (10.6)
40-49	27 (16.9)
50-59	14 (8.8)
60-69	5 (3.1)
70-79	6 (3.8)
>80	1 (0.6)

160

Acquisition of a pneumococcal type was defined as the isolation of a type for the first time in a given child. Fig. 2 shows the distribution of 336 acquisitions registered in the children (mean 2.1/child). There was a great variation in the number of different types/groups acquired by the individual children. One child was colonized with 7 different types/groups whereas no isolation of pneumococci at all was registered in 19 children.

Reacquisition was defined as the reappearance of a given type/group in a child after at least 2 routine cultures had been negative for that type/group. Among the 160 children, 24 re-acquisitions were registered. No child reacquired the same type/group more than once. Group 6 accounted for 7, 23 for 6, 19 for 5, 35 for 2 and types/groups 10, 11, 14 and 25 for one each.

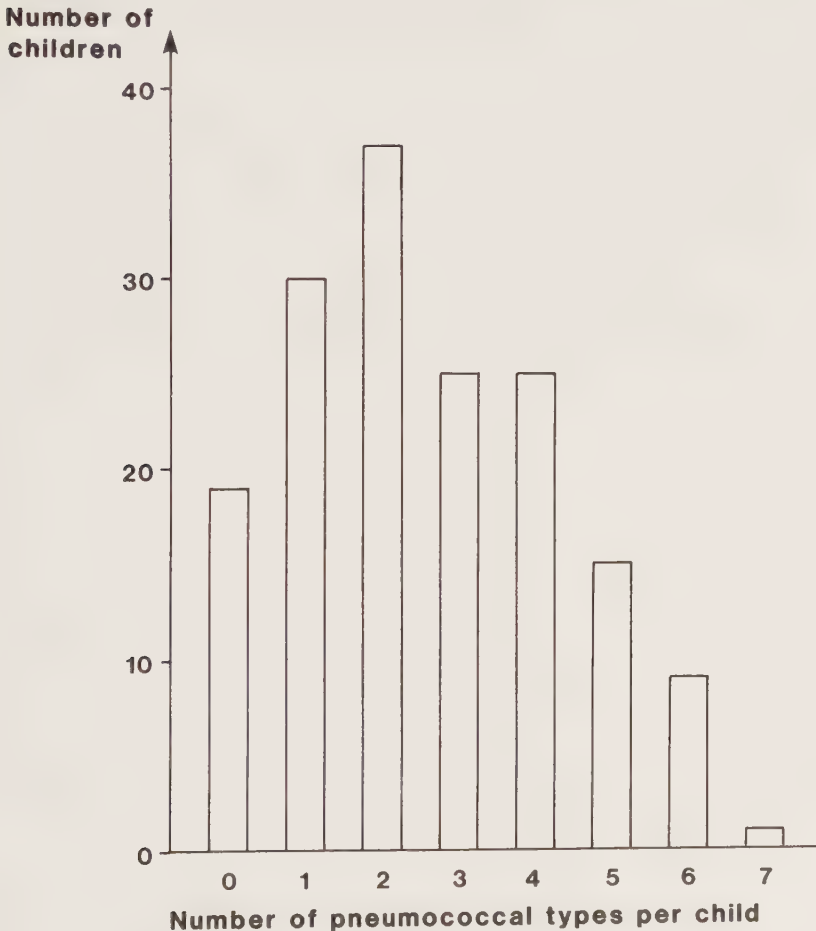


Fig. 2. Number of different pneumococcal types isolated per child in routine cultures from 160 children from whom ten or more routine cultures were obtained.

Length of carriage of the four most common pneumococcal types among the 160 children is shown in Table III. Carriage was measured from the time of acquisition to the date for the last consecutive culture that was positive for a given pneumococcal type. When a strain was recovered at only one visit the duration of carriage was assigned to one month. The types/groups 6, 14, 19 and 23 were recovered only once in 171 instances, but were carried for 3 months or more on 20 occasions.

Among the four most common types/groups, group 6 pneumococci were carried for at least 2 months in 20 of 74 cases (27%). The corresponding figures were 7/23 (30%) for type 14, 8/52 (15%) for group 19 and 8/65 for groups 23 (12%). Hence, group 6 pneumococci were carried significantly longer than groups 19 and 23 ($p < 0.05$) but not longer than type 14 ($p > 0.05$). Although the carriership for type 14 was high in per cent (30%), this type was not carried significantly longer than types/groups 19 and 23 ($p > 0.05$).

III. Relation of carriage to size of day-care centre

The total number of children attending each day-care centre during the study period varied between 12 and 96. Half of the day-care centres (33 centres) were attended by > 45 children. At most, 40% of the children at one day-care centre were enrolled in the study. 252 of the total number of 405 children attended centres with > 45 children. Among their 2,111 routine cultures, 34.2% were positive for pneumococci, in contrast to 28.7% for the children from the centres attended by < 45 children ($p < 0.01$).

Table III. *Duration of carriage of the four most common types/groups of pneumococci in 160 children cultured ten times or more with less than 45 days between each culture.*

Number of children with carriage for						
Types/Groups	1 month	2 months	3 months	4 months	5 months	Total
6	54	9	6	2	3	74
14	16	2	3		2	23
19	44	5	2		1	52
23	57	7	1			65
Total	171	23	12	2	6	

IV. Spreading within day-care centres

Spread of common types/groups of pneumococci within day-care centres was compared in those day-care centres attended by at least 12 of the children enrolled in the study. This is demonstrated in Table IV. After the first appearance of a pneumococcal type in one child, the number of children carrying that type for three consecutive months are listed. Group 6 was isolated on an average in 3.6 children in the first consecutive month after the initial appearance. The data for types/groups 14, 19 and 23 were 2.6, 2.8 and 3.2, respectively. The average number of children who carried the same type/group in the second consecutive month varied between 0.8 (group 19) and 1.5 (group 23), while data for the third month on the average ranged between 0.5 (group 23) and 0.3 (other types). No significant differences between the day-care centres could be detected.

Table IV. *Spreading within day-care centres. The number of children carrying a pneumococcal type after the first appearance of that type in one child in a day-care centre in three consecutive months. (Only day-care centres with 12 or more children participating are included; mean 13.4, range 12-16).*

Number of children with positive routine cultures after their first appearance; mean (range)			
Pneumococcal type/group	first month	second month	third month
6	3.6(1-6)	1.2(0-2)	0.3(0-2)
14	2.6(1-5)	1.1(0-3)	0.3(0.1)
19	2.8(1-4)	0.8(0-2)	0.3(0-1)
23	3.2(2-4)	1.5(1-3)	0.5(0-2)

DISCUSSION

Immunization with pneumococcal vaccine prevents pneumococcal pneumonia (17) as well as septicemia (18) in adults and reduces the number of acute otitis media episodes in children above 2 years of age (13, 19).

This study was designed as a pair-matched vaccine/placebo trial in children attending day-care centres. The environment ensured similar exposure of both vaccinees and controls to upper respiratory tract pathogens. In contrast to the procedures in other investigations, we used saline as the placebo, thereby excluding the possibility of unspecific polyclonal antibody stimulation (20, 21).

Continuous long-term data on the effect of pneumococcal vaccination on the carriership of pneumococci are sparse and results conflicting. An important aspect in the evaluation of the vaccine is whether the flora of the immunized individuals is turned towards non-vaccine types/groups, since such a change might result in an increase of infections with these types/groups. The present study which comprised the most extensive follow-up after vaccination so far, did not reveal any effect of the 14-valent vaccine on nasopharyngeal carriage of pneumococci in children attending day-care centres.

Also when group 6, known to be poorly immunogenic in children (16, 22) was excluded from the vaccine-types, no differences in isolation rates of the remaining vaccine-types/groups between vaccinees and controls were found. These results are not in disagreement with the findings of Herva and colleagues (23) and Wright and co-workers (24) who studied carriership in children at single occasions.

Herva and colleagues (23) found a slight, but not significant, reduction of vaccine-types in vaccinees, whose specimens were cultured once from the nasopharynx and nasal cavity between 6 and 12 months after pneumococcal vaccination. Wright and co-workers (24) studied children, one year of age or younger, and found that vaccination did not affect nasopharyngeal carriage of pneumococci, as examined before and after immunization.

In opposition to these data, MacLeod and co-workers (25) could report an effect of vaccination on pneumococcal carriership in the pharynx of types/groups 1, 2, 5 and 7. However, their results concerned adults who are known to respond with higher serum antibody levels after stimulation with pneumococcal polysaccharide antigens than children (26). One may speculate that a high amount of specific serum antibody, which is usually found in adults when immunized parenterally with pneumococcal polysaccharides, could be of protective value even on the nasopharyngeal mucosal level. The induction of local immunity and its relation to humoral immunity has not been clarified.

Previously we have reported (13) that children between the ages of 2 and 5 years receive benefit from vaccination with a reduction of acute otitis media episodes while no effect was seen in younger children, probably because of age-related poor immune response (16, 22).

When comparing the age-groups separately no differences in nasopharyngeal carriage of pneumococci were seen between vaccinees and controls. Pneumococci were isolated more often from children younger than 2 years of age than from the older children which is in agreement with previous reports (1, 3).

The data presented showed that day-care centres are important reservoirs of pneumococci. The four most common types/groups isolated were 6, 23, 19 and 14, which is in accordance with other studies (1, 2, 27). These are also the types/groups most frequently causing pneumococcal upper respiratory tract infections in children (8, 9, 10).

Each child acquired pneumococci in mean 2.1 times during the study period, or roughly, once a year. However, as pointed out earlier (1, 2, 28), there is a great variation in the number of pneumococcal types acquired by the individual child. Once colonized, some of the children were carriers for months. Other children reacquired the pneumococci after intervals of culture-negative specimens. These results indicate a poor immune response to pneumococci during carriage, as has also been stated by Gray and co-workers (29). Supporting this idea, group 6 - a well-known poor immunogen group (16, 22) - was carried for a significantly longer time than groups 19 och 23.

Crowding of family households has been associated with higher carriage rates of pneumococci in some studies (2, 30), but not in others (4). Day-care centres have not been investigated in this respect. Crowding was an important factor in our study. Thus, a significantly higher carriage rate of pneumococci was found among children attending more crowded day-care centres. Such a difference has also been described concerning a streptococcal epidemic at day-care centres (31). However, in our study there was no correlation between the number of family members of the participant child and carriage.

The seasonal pattern of carriage seems to be related to the degree of attendance among the children. The absence from the day-care centres due to vacations is highest in December and June-August. It might be speculated that when the children attend the day-care centres again after the vacation periods it probably takes some time before acquisition and spreading of pneumococcal types occur and this is reflected in carriage rates.

In contrast to a previous study on children attending day-care centres (1), we could often register a prompt spreading of a pneumococcal type to other children within the same centre. However, although a rather common phenomenon, this spreading seemed to be of short duration.

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Paper IV

NASOPHARYNGEAL CARRIAGE OF BACTERIA IN OTITIS-PRONE AND
NON-OTITIS-PRONE CHILDREN IN DAY-CARE CENTRES

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ABSTRACT

During a two-year period nasopharyngeal specimens were taken monthly on scheduled occasions as well as at episodes of acute otitis media (AOM) from a population of children attending day-care centres. The carriage rates of pneumococci, Haemophilus influenzae and Branhamella catarrhalis in 26 otitis-prone (OP) children when asymptomatic and at episodes of AOM were compared with the carriage rates in 36 non-otitis-prone (NOP) children. Pneumococci, H.influenzae and B.catarrhalis were found as frequently in NOP- as in asymptomatic OP-children. At AOM episodes only B.catarrhalis was found significantly more often than in the scheduled cultures. The frequencies of the six most commonly isolated pneumococcal types/groups (6, 23, 19, 14, 11, 18) were similar in the cultures taken from NOP- and OP-children on scheduled occasions as well as in cultures taken at AOM episodes.

In contrast to the NOP-children H.influenzae and B.catarrhalis were isolated less frequently in the 3-4 year-old than in the 2-3 year-old asymptomatic OP-children.

Our data indicated that the presence of pneumococci, H.influenzae or B.catarrhalis in the nasopharynx does not per se increase the risk for the development of AOM.

INTRODUCTION

Acute purulent otitis media (AOM) is a common infectious disease in childhood (1, 2, 3, 4). Pneumococci account for 30-50% of the episodes, while *Haemophilus influenzae* is responsible for approximately 20% of AOM in young children (5). Furthermore, bacteriological (6, 7, 8) as well as serological studies (9, 10) during the last two decades have shown that *Branhamella catarrhalis* causes about 10% of AOM episodes. Thus, *B.catarrhalis* nowadays is more frequently isolated in middle ear effusions in AOM than group A streptococci (11, 12).

The bacteria present in the middle ear in AOM can most frequently also be recovered from the nasopharynx (8, 13, 14) although other potentially pathogenic bacteria are also present in the nasopharynx in about half of the cases (15). Most investigators agree that nasopharyngeal cultures in AOM can help to predict the pathogen present in the middle ear (15).

On the other hand, healthy children are also frequently carriers of pneumococci, *H.influenzae* and *B.catarrhalis* (3, 16). The rate of carriage of pneumococci (17, 18) and *B.catarrhalis* is high in infancy and tends to decrease with age (3); in contrast, the rate for *H.influenzae* has been found to increase with age during childhood (3). Among the pneumococci carried in the nasopharynx, types/groups 6, 14, 19 and 23 dominate and these strains are also responsible for most middle ear infections (16, 19).

The mechanisms by which bacteria establish infections of the middle ear are not fully understood. Viral upper respiratory tract infections have been suggested to facilitate the invasion of the host by pneumococci (17, 20). The occurrence of AOM could then depend on the presence of potentially pathogenic bacteria in the nasopharynx during the trigger-infection. In consequence, children who are prone to develop AOM might carry pneumococci, *H.influenzae* or *B.catarrhalis* more frequently than non-infected children. This possibility has been proposed by some (21, 22) but others report that children without respiratory tract infections harbour pneumococci or *H.influenzae* nearly as often as children with such infections (23).

One aim of the present study was to investigate if children who are prone to develop AOM might carry the above mentioned bacteria in the nasopharynx more frequently than non-otitis-prone (NOP) children. Therefore, the nasopharyngeal carriage rate of pathogens in otitis-prone (OP) children, when asymptomatic and at AOM episodes, was compared with that in NOP-children. All the investigated children belonged to the same population of those attending day-care centres, ensuring an even exposure to upper respiratory tract infections.

MATERIALS AND METHODS

PATIENTS

The children in the present investigation were followed during their participation in a double-blind trial of a pneumococcal vaccine in day-care centres. The design of the trial has been described previously (24). In brief, 405 children were recruited after written consent was obtained from the parents. They were given Pneumovax® (Merck, Sharp and Dohme, West Point, Pennsylvania, USA) or placebo (saline with preservations).

Registrations of upper respiratory tract infections

The children were requested to attend a special clinic whenever they needed medical care. The clinic was run by two of the investigators (ENT-specialists) during the day every weekday, and at other hours by the doctor on duty at the ENT-clinic in Lund. About 10% of the patients preferred to visit their general practitioners. At each visit (both at the ENT-clinic and at the general practitioners), a special formula for registration of disease history, objective findings, diagnosis and therapy was used in addition to the usual medical record. Nasopharyngeal specimens were obtained at every visit.

Cultures on scheduled occasions from the nasopharynx

Each day-care centre attended by children enrolled in the study was visited once a month by one special nurse, who took nasopharyngeal specimens from the study participants present at the centre, provided they were not under treatment with antibiotics.

Definition of otitis-prone (OP) and non-otitis-prone (NOP) children

On the basis of the medical history before recruitment to the study, as well as the number of respiratory tract infections during the 2-year follow-up, two groups of children were selected among 253 infants born 1977-1979. These children were 1.5-3.5 years old at the onset of the study and 3.5-5.5 years old when the study was finished. Children were accepted in the OP-group if they fulfilled the criteria in one of the groups listed below:

- A) ventilation tubes due to recurrent AOM (>5 episodes) at enrollment and at least one AOM episode during the follow-up period;
- B) a history of >3 AOM episodes and during the follow-up at least 2 AOM episodes;
- C) a history of 1-2 AOM episodes and a registration of at least 3 episodes of AOM during the follow-up period.

A total of 26 (10%) of the children were regarded as OP; 14 of these infants had received Pneumovax®, the remaining saline.

Children recruited to the NOP-group had a medical history without otitis media or pneumonia, not more than one episode of acute tonsillitis or bronchitis and less than 4 episodes of common cold per year. During the 2-year follow-up period they had no episode of AOM or pneumonia, not more than one visit to a doctor and/or one period of absence from the day-care centre due to respiratory tract infection.

Thus, the NOP-children also experienced few upper respiratory tract infections.

Thirty-six children (14%) met with these demands and 23 of these children had received the vaccine. Hence, there was no significant difference between the proportion of individuals vaccinated in the OP- and the NOP-groups.

Three of the NOP-children had one course of antibiotics during the follow-up. A mean of 1.9 courses of antibiotics per OP-child was given per year during the 2-year follow-up period. Penicillin V was the drug of choice in 57% of the courses.

In the NOP-children 335 cultures, with a mean of 9.3 per child, were obtained on scheduled occasions. The OP-children, when not infected or given antibiotics, had cultures taken 268 times, 10.3 per child. Eighty-four nasopharyngeal cultures at episodes of AOM were obtained among the OP-children.

BACTERIOLOGICAL EXAMINATIONS

Pneumococci, H.influenzae, B.catarrhalis and β -haemolytic streptococci were identified according to conventional methods. Pneumococci were typed by the Quellung reaction (25). All H.influenzae and B.catarrhalis strains were investigated for betalactamase production by the use of a cephalosporin test (26).

RESULTS

Cultures on scheduled occasions

Nasopharynx specimens from NOP-children contained potentially pathogenic bacteria as often as cultures obtained from asymptomatic OP-children. The respective frequencies were 71% and 70%. The number of different pathogens recovered simultaneously in the specimen was similar in the two groups of children (Table I).

Pneumococci were isolated in 39% of the NOP-children compared with 38% in the OP-group when asymptomatic. The corresponding figures for *H.influenzae* were 34% and 35%, and for *B.catarrhalis* 34% and 39%, respectively (Fig. 1). Among the *B.catarrhalis* isolates 27% were betalactamase-producing in the OP-group when asymptomatic and 22% in the NOP-group. For *H.influenzae*, the respective proportions of betalactamase-producing bacteria were 2% in both groups.

Cultures at AOM episodes

At AOM episodes potential pathogens were found in 90% of the specimens, which was significantly more often than in cultures of scheduled occasions ($p < 0.001$) (Table I). The difference was mainly due to *B.catarrhalis* which was detected in 51% of AOM-cultures but only in 39% of cultures from asymptomatic OP-children ($p < 0.05$) and in 34% of the NOP-children ($p < 0.01$) (Fig. 1). The isolation frequencies of pneumococci (45%) and *H.influenzae* (35%) were not significantly increased at AOM (Fig. 1).

Table I. Number of pathogens recovered simultaneously in nasopharyngeal specimens from non-otitis-prone (NOP) and otitis-prone (OP) children when asymptomatic and at episodes of acute otitis media (AOM).

Number of pathogens	Number of nasopharyngeal specimens		
	NOP-children	OP-children	
	(n=335)	asymptomatic (n=268)	AOM (n=84)
1	124 (37%)	90 (34%)	41 (49%)
≥ 2	114 (34%)	96 (36%)	35 (42%)
	238 (71%)	186 (70%)	76 (91%)
No pathogens	97 (29%)	82 (31%)	8 (10%)

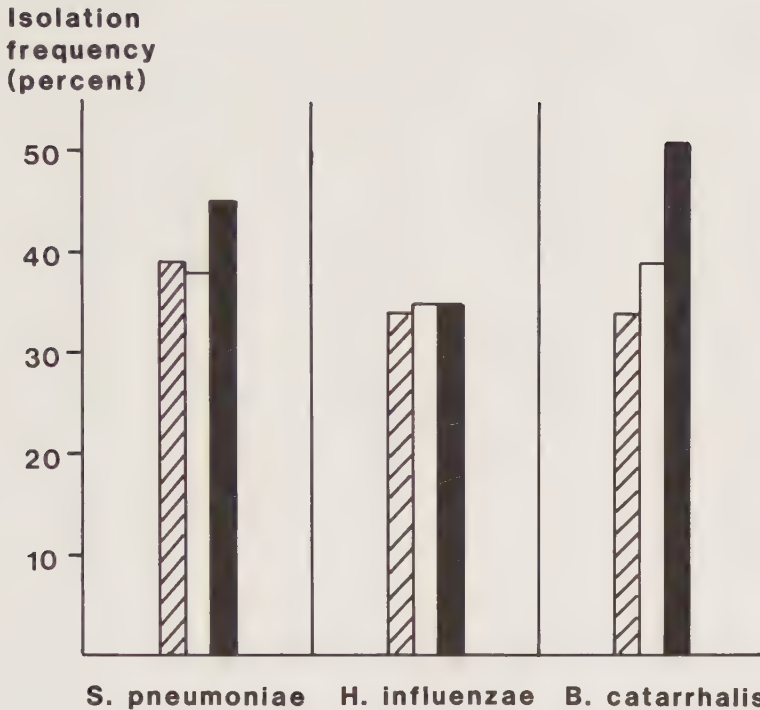


Fig. 1. Nasopharyngeal isolation frequencies of *S.pneumoniae*, *H.influenzae* and *B.catarrhalis* in 335 specimens from 36 non-otitis-prone children (▨), in 268 specimens from 26 otitis-prone children when asymptomatic (□) and in 84 specimens obtained at episodes of acute otitis media (■).

Age dependence

The cultures obtained on scheduled occasions when the children were 2-3 years old were compared with specimens collected at the age of 3-4 years (Table II). Thus, specimens taken when the children were 1.5-2 and 4-5.5 years old, respectively, were not included in this comparison.

Nasopharyngeal cultures from asymptomatic 3-4 year-old OP-children showed no pathogen in 45%, whereas the corresponding figure in asymptomatic OP-children at the age of 2-3 years was significantly lower, 22% ($p < 0.01$) as were the figures for both age groups of NOP-children, 29% and 21%, respectively ($p < 0.01$; $p < 0.01$) (Table II).

Table II. Number of pathogens recovered simultaneously in nasopharyngeal specimens from 2-3 and 3-4 year-old non-otitis-prone (NOP) and asymptomatic otitis-prone (OP) children.

Number of pathogens	Number of nasopharyngeal specimens			
	NOP children		OP children	
	2-3 yrs old (n=114)	3-4 yrs old (n=112)	2-3 yrs old (n=113)	3-4 yrs old (n=62)
1	52 (46%)	36 (32%)	41 (36%)	19 (31%)
> 2	38 (33%)	43 (38%)	47 (42%)	15 (24%)
	90 (79%)	79 (70%)	88 (78%)	34 (55%)
No pathogens	24 (21%)	33 (29%)	25 (22%)	28 (45%)

The isolation frequencies for pneumococci, *H.influenzae* and *B.catarrhalis* were similar in both age groups for NOP-children (Fig. 2). In 2-3 year-old asymptomatic OP-children, *H.influenzae* were detected in 41% and in 3-4 year-old children, in 23% ($p<0.05$). *B.catarrhalis* showed similar differences between these two age groups, 49% and 23%, respectively ($p<0.001$), whereas isolation frequencies of pneumococci were about the same (Fig. 2). In children aged 3-4 years *B.catarrhalis* was isolated, in 23% of the cultures in asymptomatic OP-children as compared with 43% in NOP-children. This difference was statistically significant ($p<0.05$).

Pneumococcal types and carriage

The six most commonly isolated types/groups of pneumococci are given in Table III. The most frequent isolate was group 6, which accounted for 29% of the pneumococci (Table III). The second and third most common groups were 23 and 19, found in 12% and 10% of the nasopharyngeal cultures, respectively. The isolation frequencies of types/groups 6, 23, 19, 14, 11 and 18 were similar in nasopharyngeal cultures taken on scheduled occasions from NOP- and OP-children when asymptomatic as well as in cultures taken at AOM episodes.

The duration of carriage for a pneumococcal strain did not differ between NOP- and OP-children. Eighteen percent of the pneumococcal strains acquired in each group were carried for more than one month. Furthermore, 3% of the strains isolated from NOP-children were carried 4 months or longer as compared to 4% of the strains in OP-children.

**Isolation
frequency
(percent)**

-9-

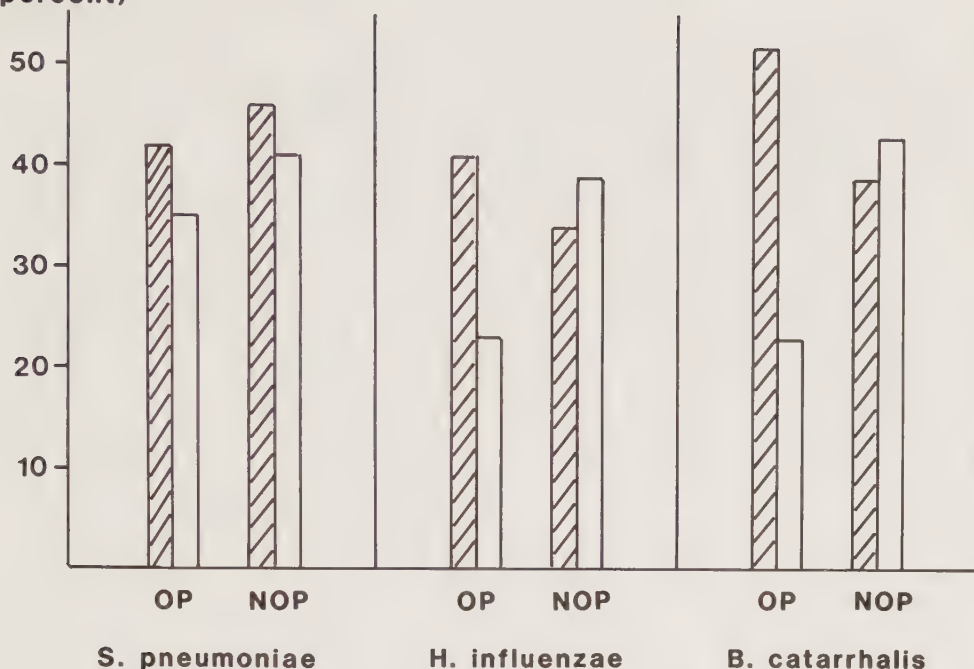


Fig. 2. Nasopharyngeal isolation frequencies of *S.pneumoniae*, *H.influenzae* and *B.catarrhalis* in 2-3 (▨) and 3-4 (□) year-old asymptomatic otitis-prone (OP) and non-otitis-prone (NOP) children. The number of nasopharyngeal specimens were 113 for 2-3 year-old and 62 for 3-4 year-old asymptomatic OP-children and 114 and 112, respectively, for the NOP-children.

Table III. The six most commonly isolated pneumococcal types/groups from the nasopharynx of 36 non-otitis-prone (NOP) and 26 otitis-prone (OP) children when asymptomatic and at episodes of acute otitis media (AOM).

Pneumococcal type/group	Total (n=269)	Number (n) of isolates (frequency %)		
		NOP (n=130)	OP asymptomatic (n=101)	OP AOM (n=38)
6	79 (29)	36 (28)	32 (32)	11 (29)
23	34 (12)	12 (9)	16 (16)	6 (13)
19	27 (10)	14 (11)	8 (8)	5 (16)
14	23 (9)	11 (8)	8 (8)	4 (11)
11	15 (6)	6 (5)	9 (9)	0 (0)
18	14 (5)	6 (5)	6 (6)	2 (5)

DISCUSSION

Pneumococci, H.influenzae and B.catarrhalis were found as frequently in non-otitis-prone (NOP) children as in the asymptomatic otitis-prone (OP) children during the two-year study period. Acquisition and duration of carriage of pneumococci were strikingly similar in the two groups. Since the children were exposed to upper respiratory tract pathogens under equivalent epidemiological conditions, our findings do not support the view that nasopharyngeal carriage of potential pathogens is more common in OP- than in healthy children, as suggested by others (21, 22). Nevertheless in an AOM episode the bacteria are considered to be derived from the nasopharynx (15). The present results indicate that host factors are crucial for the development of AOM, although the presence of a potential pathogen in the nasopharynx is a prerequisite.

That some of the children had received pneumococcal vaccine probably had no influence on the results since the proportion of vaccinees was similar among the NOP- and the OP-children. Furthermore, it has been demonstrated that pneumococcal vaccination does not change the nasopharyngeal carriage of bacteria (27, 28). It is not likely that treatment with antibiotics in the OP-group to any large degree contributed to the results by eradicating the bacteria from the nasopharynx. The effect of antibiotics is only transient (29) and cultures were not taken during the courses. The number of courses of antibiotics amounted to 1.9/child-year in the OP-group. Moreover, the proportions of betalactamase-producing bacteria were similar in the two groups, although penicillin was mostly used in the treatment of infection in the OP-children.

Among the pneumococci, types/groups 6, 14, 19 and 23 are most frequently involved in AOM (5, 30). The isolation frequencies of these types/groups did not differ between NOP- and OP-children, whether asymptomatic or at AOM episodes. Our results suggest that the frequency with which different pneumococci cause AOM is proportional to the rates of occurrence in the nasopharynx of children. The finding by Austrian and co-workers (31) and Gray and colleagues (16) that type 14 was more frequently found in AOM-effusions than among healthy nasopharyngeal carriers was not corroborated by the present findings. Although we compared nasopharynx specimens, our isolation frequency for type 14 was quite similar to that obtained in middle ear effusions in another Scandinavian investigation on AOM (32). Thus, the typing data also pointed to the importance of host factors in the development of AOM and did not support the existence of specific "otitis-strains" among the pneumococci harboured in the nasopharynx.

The carrier rates of pneumococci, H.influenzae and B.catarrhalis among the NOP-children were similar whether the children were 2-3 or 3-4 years old which is consistent with

the findings of Ingvarsson and colleagues (3). In contrast, carriage of H.influenzae and B.catarrhalis was less frequent in the age period between 3-4 years compared with 2-3 years in asymptomatic OP-children, which could indicate that the children had developed immunity to these bacteria. Regarding pneumococci, no decrease in carriage was seen during the fourth year, which is consistent with a delay in immune-responsiveness to pneumococcal capsular polysaccharides. Such a delay is seen in many healthy children (33) but is most pronounced in children with recurrent AOM (34).

B.catarrhalis was the only pathogen that could be isolated more frequently from the nasopharynx at AOM episodes, a possible explanation might be that the viral "trigger"-infection (17, 20) in AOM facilitates colonization of the mucosa with these bacteria.

In conclusion, in day-care centres, NOP-children carry potentially pathogenic bacteria in the nasopharynx as frequently as OP-children. Thus, the presence of these bacteria in this site does not per se increase the risk for development of AOM.

ACKNOWLEDGEMENTS

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Paper V

Opsonic and Antibody Responses to Pneumococcal Polysaccharide Types 6A, 19F and 23F after Vaccination of Immunocompromised Patients

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Opsonic and antibody responses to pneumococcal polysaccharide types 6A, 19F and 23F were evaluated before and after vaccination with a 14-valent pneumococcal vaccine in 25 patients splenectomized due to trauma, non-malignant or malignant disease and in 8 non-splenectomized patients with malignant disease. In approximately 50 % of the tests, a 2-fold or greater increase in antibody concentrations and a significantly enhanced opsonization of pneumococci was found. A close correlation between antibody increase and enhancement of opsonization was demonstrated. 93 % of paired samples with postimmunization antibody increase above 150 ELISA units showed significantly enhanced opsonization. Increased postvaccination opsonic activity and antibody levels were infrequently accompanied by increased granulocyte chemotactic activity of the serum. No significant difference in antibody and opsonic response to vaccination was found between the groups of patients, except for patients with Hodgkin's disease receiving chemotherapy, who had a reduced immunization response. Prevacination antibody concentration, type of antigen or age of the patients did not influence the outcome of vaccination.

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INTRODUCTION

Antipneumococcal antibodies promote phagocytosis (opsonization) and subsequent killing of pneumococci via formation of antigen-antibody complexes, which bind and activate the complement system (27).

Pneumococcal polysaccharide vaccine is immunogenic and affords protection against infection with vaccine type pneumococci in young healthy adults (3, 25). The antibody response to pneumococcal vaccination in various immunocompromised patients, known to be at high risk of pneumococcal infection, has been found reduced, however (9, 14, 21, 22, 24). Furthermore, 25–40 % of patients with a significant antibody response after vaccination have not demonstrated enhancement of type specific opsonization (9, 12). Conclusive studies of clinical protection by the vaccine in high risk groups are not available (1, 8). Therefore, evaluation of vaccine efficacy presently must be based upon studies of serological response, and on knowledge about the correlation between increased antibody concentration and enhanced opsonization.

The purpose of the present investigation was to study serum opsonic activity and antibody responses to 3 pneumococcal polysaccharide antigens of different immunogenicity (10), after vaccination with a 14-valent pneumococcal vaccine of different groups of patients with high risk for pneumococcal infection.

MATERIAL AND METHODS

Bacteria. *Streptococcus pneumoniae* serotypes 6A, 19F and 23F were obtained from Statens Serum-institut, Copenhagen, Denmark. The pneumococci were maintained on blood agar and the strains were renewed monthly from stock cultures in foetal calf serum at -80°C . The bacteria were cultured for 4 h in a brain heart infusion broth (Difco Laboratories, Detroit, USA) supplemented with 50 mg/ml cysteine chloride in an anaerobic environment (GasPak, Becton, Dickinson and Co, Cockeysville, USA) and harvested in the logarithmic growth phase, washed and suspended in Hanks' balanced salt solution (HBSS) buffered with 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid), pH 7.4 as earlier described (6).

Patients. The following groups of patients were studied: (1) 8 individuals splenectomized due to trauma (mean age 29.4 yr); (2) 9 patients splenectomized because of non-malignant diseases (leukopenia, thrombocytopenia, spherocytosis, myelofibrosis and pancreatitis) (mean age 49.5 yr); (3) 8 patients splenectomized due to malignant diseases of the hematopoietic system, 5 with Hodgkin's disease and 3 with hairy cell leukemia (mean age 52.6 yr); and (4) 8 non-splenectomized patients with malignant hematopoietic diseases, 7 with Hodgkin's disease and 1 with non-Hodgkin lymphoma (mean age 42.5 yr).

Granulocytes. Granulocytes from healthy individuals were isolated from peripheral blood using Isopaque-Ficoll. After lysis of red cells with 0.87% NH_4Cl , the granulocytes were washed and suspended in HEPES-HBSS as earlier described (20).

Sera. Sera were collected before and 1.4–2.5 months (mean for groups) after immunization with a 14-valent pneumococcal vaccine (Pneumovax, Merck Sharp & Dohme, West Point, USA) containing polysaccharide types 1, 2, 3, 4, 6A, 7F, 8, 9N, 12F, 14, 18C, 19F, 23F and 25. The sera were stored in aliquots at -80°C .

Antibody determinations. Type specific anti-pneumococcal polysaccharide antibodies measured as total immunoglobulin were determined by enzyme-linked immunosorbent assay (ELISA) as earlier described (13).

Phagocytic killing of bacteria. Opsonization was measured as granulocyte phagocytic killing of pneumococci, since ingested *S. pneumoniae* are rapidly killed by granulocytes (6). Approximately 5×10^6 organisms were incubated for 30 min at 37°C with 10^6 granulocytes in serum diluted to a final concentration of 16%, in a total volume of 250 μl . Surviving microorganisms were measured by colony counting as previously described (6). If bacterial killing at 16% serum was less than 20% or greater than 80% in both pre- and postvaccination samples, the serum pair was retested at 32 and 8% concentrations, respectively. The variation of the assay system was evaluated at different levels of bacterial killing. The SD at 20% bacterial killing was $\pm 4.6\%$ and at 80% killing $\pm 6.3\%$. Increased opsonic activity in postvaccination sera was defined as an increase in bacterial killing exceeding 2 SD.

Some patient sera were heat inactivated at 56°C for 30 min and tested with serum from a patient with selective and complete deficiency of IgG subclass 2 (IgG2) as a complement source (IgG subclass determination kindly performed by Dr V-A Oxelius, Lund). The IgG2 deficient serum had normal levels of C3, C4, C5, factor B and properdin (kindly determined by Dr A. Sjöholm, Lund) and was free of antibodies to pneumococcal polysaccharides types 6A, 19F and 23F measured with ELISA.

Granulocyte chemotaxis. The chemotaxis of granulocytes from healthy individuals was studied on agarose plates with a method modified from Nelson et al. (19). Approximately 10^9 *S. pneumoniae* serotypes 6A, 19F and 23F were incubated in 12.5% of pre- or postvaccination serum for 4 min at 37°C . A serum concentration of 12.5% had been found suitable, since it supported a substantial but suboptimum chemotaxis. After heat inactivation (56°C , 30 min) followed by centrifugation, the supernatants were used as chemoattractants. 1×10^6 granulocytes migrated under agar towards wells containing 10 μl of chemoattractant. Incubations were for 3 h at 37°C in a humidified atmosphere. The under-agarose technique measures simultaneously the random migration towards wells containing control medium (distance B) and the distance covered by the cells in response to a chemoattractant (distance A). The chemotactic index was expressed by the ratio A/B. The SD of the method for experiments performed simultaneously was 5%.

Statistical calculations. Comparison of the numbers of patients responding in each group and to each serotype was done by means of χ^2 -test or Fisher's exact test.

RESULTS

The serum opsonic activity and the arbitrary concentration in ELISA units of antibodies against *S. pneumoniae* serotypes 6A, 19F and 23F in the 4 groups of patients before and after vaccination are shown in Figs. 1–4. Pneumococcal vaccination induced increased

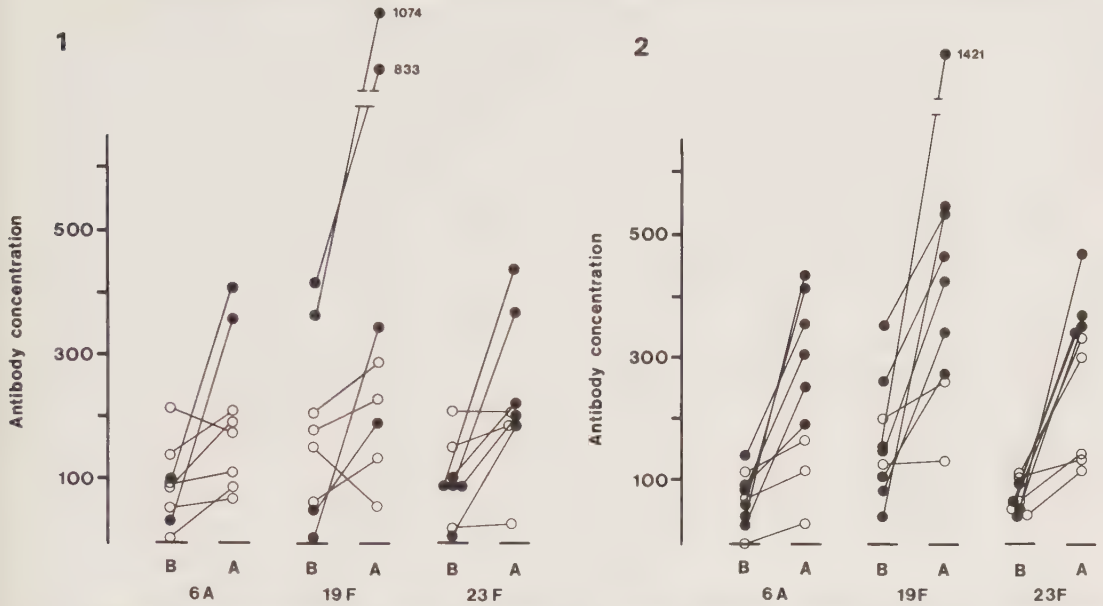


Fig. 1. Antibody concentration and opsonic response to *S. pneumoniae* serotypes 6A, 19F and 23F in patients splenectomized due to trauma before (B) and after (A) immunization with a 14-valent pneumococcal vaccine. Antibody concentration, measured with ELISA, is expressed in arbitrary units. ● = significant increase of pneumococcal opsonization after immunization; ○ = unchanged opsonic activity.

Fig. 2. Patients splenectomized due to non-malignant diseases. Symbols are identical with those of Fig. 1.

opsonization of serotypes 6A and 23F in 47% each and of serotype 19F in 63% of the patients ($p>0.05$). The antibody concentrations rose more than 2-fold against serotypes 6A, 19F and 23F in 58%, 53% and 56%, respectively, of the patients. A close correlation was found between antibody increase and enhancement of opsonization. Serum pairs with antibody concentration increases of >150 arbitrary units showed significantly enhanced opsonization in 93% (43/46) of the assays, while only 13% (6/48) with antibody concentration increase <100 units were associated with increased opsonization. In sera with at least 2-fold antibody rise after vaccination, 83% (45/54) of the assays showed increased opsonic activity.

Five serum pairs with discordance between opsonic and antibody response to vaccination, were also tested for opsonic activity after heat-inactivation and with replacement of complement by addition of non-immune serum. Identical results with those of the non-inactivated sera were found.

No difference in the response to immunization among patients with low (≤ 50 units) or high (≥ 200 units) prevaccination antibody concentrations was found. In serum pairs with low prevaccination antibody levels, increased opsonic activity and 2-fold or greater antibody rise were found in 56 and 59% respectively, of the assays. In serum pairs with high prevaccination levels, the corresponding figures were 59 and 50% respectively. High prevaccination antibody levels were found more often against serotype 19F than against 6A and 23F ($p<0.05$).

The underlying disease had little influence on the outcome of vaccination. Postvaccination sera showed in the different groups of patients increased opsonic activity in 46–63% of the assays and at least 2-fold antibody rise in 50–63% (Figs. 1–4). These differences between the various groups of patients were not significant. In 7 patients splenectomized

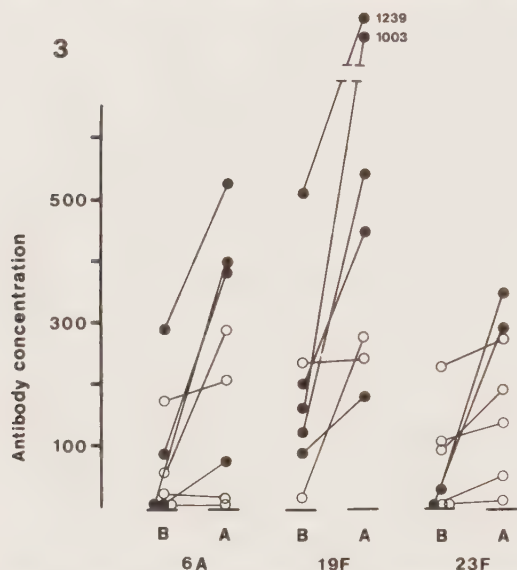


Fig. 3. Patients splenectomized due to malignant diseases. Symbols are identical with those of Fig. 1.

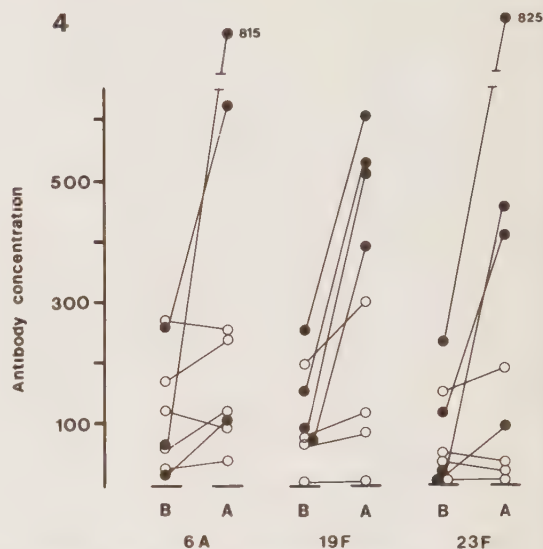


Fig. 4. Non-splenectomized patients with malignant diseases. Symbols are identical with those of Fig. 1.

less than a year before vaccination, increased opsonization was found in 76% (16/21) of the assays. In 18 patients vaccinated more than a year after the splenectomy, 48% (26/54) showed increased opsonization ($p < 0.05$). A 2-fold antibody concentration increase was found in 81% of the assays with serum pairs from patients with less than a year's interval between vaccination and splenectomy and in 46% of assays with serum from patients with more than a year's interval ($p < 0.01$).

Advanced age was well compatible with a favourable outcome of vaccination. In 24 assays from 8 patients > 60 yr old, increased opsonization was found in 67% and a more than 2-fold antibody increase in 52%.

Five patients with Hodgkin's disease were vaccinated and studied during chemotherapy, and 2 begun chemotherapy soon after immunization, before the postvaccination sample was drawn. Among these patients increased opsonization was found in 24% (5/21) of the assays and, a 2-fold or greater antibody increase in 33%, which is significantly lower than in the remainder of our patients ($p < 0.01$ and $p < 0.05$, respectively). Two other patients with Hodgkin's disease, vaccinated during radiotherapy responded with increased opsonic activity and 2-fold antibody increase in 6/6 experiments.

Chemotactic activity was studied in serum pairs from 5 patients with increased opsonic activity and at least 2-fold increase of antibody concentration after vaccination. No increase of granulocyte chemotaxis was induced by *S. pneumoniae* serotype 6A and postvaccination sera. In experiments with serotypes 19F and 23F increased chemotaxis was found in 2 and 1 postvaccination sera, respectively.

DISCUSSION

Phagocytosis is essential for the human defence against *S. pneumoniae* (27). The capsule of the pneumococcus has antiphagocytic properties (17) and effective opsonization of this organism requires activation of the complement factor C3 by specific anti-capsular antibodies (7).

In the present study, it was found that approximately 50% of patients with high risk of pneumococcal infection, after immunization showed at least 2-fold antibody increase and enhanced opsonic activity against the tested pneumococcal serotypes. A close correlation between antibody increase and enhancement of opsonization was found, provided that increase in antibody concentration was above 150 ELISA units. A similar correlation was not found for antibody increase below that value, which only may be a reflexion of the sensitivity of the opsonic assay. In a recent study it was estimated that the mean, minimum pneumococcal antibody concentration conferring immunity against 6 pneumococcal serotypes was about 130 ELISA units (16). This is about the same concentration as we found required for demonstration of improved opsonization.

Giebink et al. (9) studied the opsonic activity after vaccination against serotypes 3, 6A and 7F in children splenectomized due to traumatic splenic laceration. 42% of the children with at least 2-fold antibody increase after vaccination failed to show an autologous opsonic response. Simberkoff et al. (24) found, after vaccination, increased opsonic activity against serotypes 1 and 3 in only 50 and 36% of adult chronic hemodialysis patients. A poor correlation between postvaccination antibody levels and opsonic activity was found for serotype 1. Also Johansen and Karup Pedersen (12) found, in a study of splenectomized individuals and healthy adults, that 30% of patients with a significant response to vaccination did not display any increase in type specific opsonization.

Much of the discrepancies in opsonic activity between these and our investigations are likely to be methodological. The outcome of the phagocytic assay used in our study to evaluate the opsonic activity of serum is dependent on the concentration of the serum employed (6). In the present investigation the opsonic activity has been evaluated with serum concentrations which supported a moderately efficient phagocytic killing (20–80%) of the pneumococci. Simberkoff et al. (24) imposed a high request on increase in opsonic activity after immunization and the serotypes used in both their and the Danish (12) study, particularly serotype 3, are provided with an unusually large antiphagocytic capsule (4). The low number of increased opsonic activity in assays after vaccination, found by Giebink et al. (9) is probably due to the use of only one serum concentration.

The similar immunogenicity of type antigens 6A, 19F and 23F, contrasting to other studies (10, 15), could be due to the large individual variation in response to polysaccharide antigens (15), rendering conclusions less reliable with the small groups studied. This might also explain the discrepancy between our finding of a more favourable outcome of vaccination when performed less than a year after splenectomy and the observations by Giebink et al. (9). They found no difference in response to pneumococcal vaccination between 10 patients immunized within 4 weeks of splenectomy and 5 children vaccinated about 5 yr after splenectomy. Advanced age did not influence the result of pneumococcal vaccination. Similar observations have been made by Hilleman et al. (10).

The impaired antibody response after pneumococcal vaccination of patients with Hodgkin's disease treated with radiation and chemotherapy is in agreement with other studies (2, 18, 22). It has therefore been recommended that pneumococcal vaccination is performed either before or some time after treatment for Hodgkin's disease (23).

The main role of antibody in opsonization of *S. pneumoniae* is probably to mediate the fixation of opsonic complement, C3b, to the bacterial surface (7). During the further activation of the complement system, C5a, the major chemoattractant of serum is formed (26). In the present study 5 serum pairs with increased opsonic activity and antibody concentrations were evaluated for granulocyte chemotactic activity. The results indicate that increased levels of opsonic antibodies after pneumococcal vaccination are not regularly accompanied by increased granulocyte chemotaxis.

Antibodies in this study are measured as total immunoglobulin, including non-opsoniz-

ing IgA antibodies. Our results, which indicate a good correlation between opsonic activity and ELISA-measured antibodies, thus suggest that the major part of the antibodies found after vaccination are of IgG and of IgM class, as suggested by others (5, 11, 15). The correlation between anti-pneumococcal antibody and opsonic activity makes judgement of vaccine efficacy based upon the simpler antibody determinations possible.

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